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Response of Vegetation to Carbon Dioxide

043

Response of Rice to Subambient and Superambient Carbon Dioxide Concentrations 1986-1987



Response

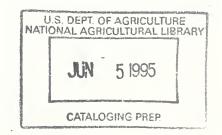
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Carbon Dioxide Concentrations

1986-1987



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Progress Report of Research: RESPONSE OF RICE TO

SUBAMBIENT AND SUPERAMBIENT CARBON DIOXIDE CONCENTRATIONS. 1986-1987 Progress Report.

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Project Title: ASSESMENT OF CROP RESPONSE TO INCREASED ATMOSPHERIC CARBON DIOXIDE: RISING CO₂ EFFECTS ON RICE

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DISCLAIMER

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This progress report was prepared in partial fulfilment of requirements of the Interagency Agreement, and does not represent formal publication in the open literature. Much of the information contained herein will be reported later in formal refereed journal publications.

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8

TABLE OF CONTENTS

		<u>Page</u>	
TITL	E PA	GE:	i
INVE	STIG	ATORS i:	i
PROGI	RAM	STRUCTURE iii	i
ACKNO	OWLE	DGEMENTS i	V
TABL	E OF	CONTENTS	V
SECT	ION	I.	
I.	EXE	CUTIVE SUMMARY	1
	A.	Introduction	1
	в.	Rationale	2
	c.	Approach	4
	D.	Salient Findings	9
	E.	Literature Cited	5
SECT	ION	II.	
II.		elopmental Responses of Rice to Photoperiod Carbon Dioxide Concentration	
	A.	Abstract 16	
	в.	Introduction	
	c.	Materials and Methods 20	
	D.	Results and Discussion 26	
	E.	Literature Cited	
SECT	ION	III.	
III.	Amb	wth and Yield Responses of Rice to Subambient, ient, and Superambient Carbon Dioxide centrations	
	Α.	Abstract 37	

U

	В.	Introduction	. 39
	C.	Materials and Methods	42
	D.	Results and Discussion	. 47
	E.	Literature Cited	, 74
SECT	ION :	IV.	
IV.	Res	e Photosynthesis and Evapotranspiration ponses to Subambient, Ambient, and Superambient bon Dioxide Concentrations	
	A.	Abstract	. 79
	В.	Introduction	81
	C.	Materials and Methods	. 82
	D.	Results	. 87
	E.	Discussion	100
	F.	Literature Cited	105
SECT	ION '	V.	
V.	Var	nges in Stomatal Density in Rice Grown Under ious CO ₂ Regimes with Natural Solar adiance	108
	A.	Abstract	108
	в.	Introduction	110
	c.	Materials and Methods	112
	D.	Results and Discussion	114
	E.	Literature Cited	123
SECT	ON '	VI.	
VI.		isco Activity and Rubisco Protein Content in e Grown under Various CO ₂ Concentrations	127
	A.	Abstract	127
	B.	Introduction	129

	1
	1
	1

	C.	Materials and Methods	131
	D.	Results	134
	E.	Discussion	143
	F.	Literature Cited	150
SECT	ION '	VII.	
VII.	Seas	ect of CO ₂ Concentration During the Growing son on Carbohydrate Status and Partitioning Rice	156
	A.	Abstract	156
	в.	Introduction	158
	c.	Materials and Methods	160
	D.	Results	164
	Ε.	Discussion	188
	F.	Literature Cited	198
SECT	ON '	VIII.	
VIII		enetically Altered Cyanobacteria as Nitrogen ertilizer Supplier for Growth of Rice	204
	A.	Introduction	204
	В.	Ammonia Assimilation	205
-	c.	Excreation of Ammonia by Mutant	208
	D.	Glutamine Synthetase	214
	E.	Rice: Cyanobacteria Interactions	216
	F.	References	229
SECT	ION :	IX.	
]	PROC	ICATIONS: TECHNICAL REPORTS, JOURNALS OR EEDINGS, AND PUBLISHED ABSTRACTS	233

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SECTION I EXECUTIVE SUMMARY

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I. EXECUTIVE SUMMARY

INTRODUCTION

This report, number 043 of the series: Response of Vegetation to Carbon Dioxide, summarizes the results of experiments conducted in 1987 at Gainesville, Florida on rice. Other reports in this series from this location are Series No. 003, 007, 014, 031, and 032 with soybean as the crop.

experiments were conducted in These computercontrolled, naturally sun-lit, plant growth chambers commonly referred to as SPAR (Soil Plant Atmosphere Research) The conversion from soybean to flooded paddy required extensive modifications to the SPAR units. soil lysimeter section of each SPAR unit was fitted with a water tight aluminum vat filled with soil in order to provide a flooded soil environment for growing rice paddy culture. Since rice is very responsive to the temperature of the flood water, it was further necessary to develop a paddy water temperature control subsystem for each SPAR unit.

Two experiments, each covering the complete growing season from planting to harvest, were conducted on rice in these modified SPAR units in 1987. The CO₂ treatments were identical in both experiments and consisted of subambient (160 and 250 ppm), ambient (330 ppm), and superambient (500, 660 and 900 ppm) CO₂ treatments.

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RATIONALE

The reasons for selecting the ambient (330 ppm) and twice ambient (660 ppm) CO₂ treatments are self explanatory in light of the expected doubling of current levels of atmospheric CO₂ projected to occur sometime during the next century (Edmonds et al., 1984; Trabalka et al., 1986). However, the rationale behind the selection of subambient CO₂ treatments may not be as obvious initially.

Based on gasses trapped in ice cores from the Antarctic and Greenland, Barnola et al. (1983) have estimated that the atmospheric CO₂ concentration may have been as low as 180 ppm 15,000 to 20,000 years ago. Ice core studies have placed the atmospheric CO₂ concentration at around 280 ppm, by the end of the last ice age (Berner et al., 1980; Delmas et al. 1980; Neftel et al., 1982). Based on the results of Neftel et al. (1985) and findings from other ice core studies (Barnola et al., 1983; Raynaud and Barnola, 1985), Gammon and Fraser (1985) estimate the 1880 or preindustrial CO₂ concentration at 265 to 285 ppm; a range of values little changed since the end of the last ice age.

Reasons for including the subambient treatments are two-fold. First, as previously noted, CO_2 concentration of the earth's atmosphere was considerably lower in the past. The response of C_3 plants to subambient levels is therefore of historical as well as physiological interest. Second, nonlinear models of plant responses to CO_2 concentration show the greatest amount of curvature in plant response at

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subambient ${\rm CO}_2$ concentrations (Allen et al., 1987). Thus, the subambient treatments were included in order to provide a more complete plant response surface to ${\rm CO}_2$ concentration.

Finally, we chose 330 ppm as the ambient level of ${\rm CO}_2$ concentration to maintain a fixed control level (representative of global ${\rm CO}_2$ concentrations in 1973). This avoids having a continuously varying level of ${\rm CO}_2$ from year-to-year as atmospheric ${\rm CO}_2$ concentration gradually rises.

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APPROACH

Controlled Environment Chambers

Rice plants were grown season long in six outdoor, controlled environment plant growth chambers. These chambers are exposed to natural sunlight and are constructed of a clear cellulose acetate roof with mylar walls. Above ground chamber dimensions are 2.0 x 1.0 m in cross section by 1.5 m in height. These chamber tops were attached to an aluminum vat, filled with soil and measuring 1.5 x 0.8 m in cross section and 0.5 m deep in order to provide a water tight, flooded root environment for growing rice in paddy culture.

A dedicated computer operating in real time was used to maintain environmental control in each of the chambers and record plant response and environmental data to magnetic disc every 5 min. Signals from an array of sensors in each chamber were monitored at specific time intervals and used in a variety of control algorithms to actuate various devices in order to maintain desired environmental control.

Dewpoint temperature was controlled to 18°C by driving a bypass valve which determined the rate of flow of chilled water through cooling coils. The condensate from the cooling coils passed through tipping bucket rain gauges monitored by the computer to calculate evapotranspiration rate over 5-min intervals. Dry bulb air temperature was controlled to 31°C by turning on and off electrical resistance heaters. Daytime carbon dioxide concentration was

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maintained at 160 and 250 ppm (subambient CO₂ treatments), 330 ppm (ambient CO₂ treatment), 500, 660 and 900 ppm (superambient CO₂ treatments) using a solenoid valve actuated, a CO₂ injection system. Carbon dioxide was injected from high pressure cylinders in order to replace the CO₂ taken up by the plants during photosynthesis. Canopy photosynthetic rate was calculated from CO₂ mass balances over each 5-min interval. A similar control algorithm was used to maintain subambient CO₂ levels by injecting CO₂-free air into the subambient chambers during periods of low photosynthetic rates experienced early in the season prior to canopy closure or during periods of low light intensity.

Paddy flood water depth was maintained at 50 mm above the soil surface using a float actuated watering valve. Flood water temperature was maintained between 27 and 29°C using resistance heaters consisting of plastic coated nichrome heating wire wrapped around nine 0.8 m long aluminum rods and placed between the rows within the flood water.

In order to compare the developmental responses of rice to CO₂ concentrations under different photoperiods, the photoperiod was extended during the EPR experiment with four supplemental, incandescent, 75 watt lights from 1700 to 2400 Eastern Standard Time (EST) until 2 Mar. 1987.

Plant Culture

The experiment was conducted twice during 1987 with

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planting dates of 22 Jan., for the early planted rice (EPR) experiment and 23 June for the late planted rice (LPR) experiment. In both experiments, rice was direct seeded by hand, into 11 rows in each chamber, 0.18 m apart. The plants were thinned to 235 plants m⁻² and flood water was applied at the second leaf stage in both growing seasons. Shades were maintained at canopy height along the outside of each chamber in order to provide a light environment found in a field crop.

Immediately prior to the application of the flood, the soil in each chamber was fertilized with N, P, and K at rates of 12, 11, 11 and 10, 9, 9 g m⁻² for the EPR and LPR, respectively. In all cases, fertilizer N was applied as N-15 enriched urea. Additional N was applied at rates of 4.5, 4.5, and 8.0 g m⁻² at 32, 51, and 61 days after planting (DAP), respectively for the EPR and at rates of 4.8, 4.8, and 9.5 g m⁻² at 23, 42, and 64 DAP, respectively for the LPR. The soils used were a Chandler fine sand and a Zolfo fine sand for the EPR and LPR, respectively.

Development, Growth, and Yield Measurements

The main stems of five replicate plants in each chamber were tagged with a small plastic loop prior to flooding in both experiments. Stage of leaf development was determined for these mainstems two to three times a week. Dates for panicle initiation were determined twice a week by dissection of mainstems from five randomly sampled plants from each chamber. The developing panicles at this point

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were 1 to 4 mm in length measuring from the developing neck node to the tip of the panicle. Heading and physiological maturity were evaluated on a canopy basis in both experiments. In the LPR experiment, plants were destructively sampled at 19, 30, 44, 58, 71, 86, and 110 days after planting. For each sampled plant the number of living leaves, tillers and panicles, were counted and lear area per plant was measured. Dry weights were determined for each plant part after oven drying. Final grain yield was determined for both experiments at the end of the growing season.

Stomatal Density

In order to determine the season-long effects of CO₂ concentration on leaf morphology, stomatal density (number of leaf stomata/unit leaf area) was determined in the LPR experiment. Clear varnish imprints of the 7th mainstem leaf and the flag or last mainstem leaf were made on plants in each treatment. From these imprints the number of stomata on the upper and lower leaf surfaces were counted and stomatal densities for each CO₂ treatment were calculated.

Rubisco Activity and Carbohydrate analyses

In the LPR experiment, leaf samples were collected at mid-day from all six ${\rm CO}_2$ treatments and immediately frozen in liquid nitrogen. These samples were then assayed for Rubisco (carboxylase) activity. In order to determine the

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daytime trends in Rubisco activity as affected by CO₂ treatment, similar assays were conducted from dawn to dusk for the 160, 330, and 660 ppm CO₂ treatments. Total non-structural carbohydrate concentration, starch concentration, and sugar concentrations in the glucose, sucrose, fructose forms were determined for the plant parts destructively sampled during the LPR experiment.

Plant and Soil Nitrogen

Plant, soil, and soil water samples were taken for total nitrogen analyses and N-15 mass spectrometer analyses. Part of the objective was to determine the losses of fertilizer N under different CO₂ regimes at the specified temperatures used in the study as well as to determine N content of the plant tissue. The samples have not been analyzed yet. We are waiting for new equipment to arrive at the University of Florida.

SALIENT FINDINGS

Development

- 1. In both experiments, leaf developmental rates during the vegetative phases of growth were almost double that of the reproductive phase of growth.
- 2. In both experiments, with increasing CO₂ concentration, leaf developmental rates during the vegetative phase of growth were increased slightly while leaf developmental rates during the reproductive phase of growth were unaffected by CO₂ treatment.
- 3. In the LPR experiment, panicle initiation, and boot stage occurred earlier and total growth duration was shortened for plants in the superambient compared with ambient and subambient treatments.
- 4. The acceleration of plant development with increasing CO_2 concentration in the LPR experiment was associated with a CO_2 induced reduction in the duration of the vegetative phase of growth and a decrease in the number of main stem leaves formed during vegetative growth.
- 5. The artifical lights used to extend the photoperiod during vegetative development in the EPR experiment forced a delay in the onset of reproductive development, especially in the superambient treatments. This resulted in no difference among the CO₂ treatments in total growth duration, the timing of physiological growth stages, or in the number of mainstem leaves produced in the EPR experiment.

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Growth and Grain Yield

- 1. Total biomass, biomass of individual plant parts including the roots, tillering, and final grain yield followed a similar pattern with increasing CO₂ treatment: an increase in growth and yield response from the 160 to the 500 ppm treatments followed by a leveling of the response 500 ppm treatments followed by a leveling of the response across the superambient treatments.
- 2. The partitioning of biomass between the root and shoots was most affected by CO_2 treatment early in the growing season with root:total plant dry weight ratios increasing with increasing CO_2 treatment. Root:total plant dry weight ratios decreased during the growing season in all CO_2 treatments.
- 3. Differences in biomass and leaf area among the CO_2 treatments were largely due to corresponding differences in tillering response. However, biomass was affected to a far greater degree by CO_2 than was leaf area.
- 4. The number of panicles per plant was the yield component almost entirely responsible for differences in final grain yield among the ${\rm CO}_2$ treatments.
- 5. Tiller numbers increased at the end of the growing season due to the production of small ineffective tillers during and following seed fill. The relatively greater end-of-season tillering response of the superambient compared with the subambient treatments suggests a greater rationing ability of the CO₂ enriched plants.

resulted in a 38% increase in grain yield while the 160 ppm ${\rm CO}_2$ treatment resulted in a 43% reduction in grain yield compared to the 330 ppm ${\rm CO}_2$ treatment.

Photosynthesis and Evapotranspiration

1. Prior to the beginning of stem extension the gross photosynthetic light response was adequately described by a rectangular hyperbola of the form:

$$P_{cl} = \alpha I \tau C / (\alpha I + \tau C)$$

where P_g is gross photosynthesis (eg. net photosynthesis (P_n) + dark respiration (R_d)), α = canopy light utilization efficiency, I = photosynthetic photon flux density, τ = canopy conductance to CO_2 transfer, and C = external CO_2 concentration.

- 2. Estimates of α , the initial slope of the light response curve near the origin, increased with increasing ${\rm CO}_2$ with the greatest amount of increase occurring from the 160 to the 500 ppm ${\rm CO}_2$ treatments.
- 3. Estimates of τ were far more variable than those for α and were rarely significantly different among ${\rm CO_2}$ treatments.
- 4. After the beginning of stem extension, the canopy photosynthetic light response became linear and showed little tendency towards light saturation.
- 5. Photosynthetic rates increased with increasing ${\rm CO}_2$ treatment from the 160 to the 500 ppm treatments followed by a leveling off of the response among the superambient

treatments.

- 6. Short term cross-switching of ${\rm CO_2}$ concentrations among the chambers revealed a profound adaptive response to ${\rm CO_2}$ acclimation treatment: photosynthetic rate, measured at a common external ${\rm CO_2}$ concentration, decreased with increasing ${\rm CO_2}$ acclimation treatment.
- 7. Evapotranspiration decreased while water-use efficiency increased with increasing CO₂ concentration.

Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco)

- 1. Rubisco activity decreased with increasing CO₂ concentration.
- 2. This drop in Rubisco activity was accompanied by a decrease in the concentration of leaf Rubisco protein, expressed in terms of total soluble protein content.
- 3. Dark inhibition of Rubisco was found to take place in rice. Species exhibiting this inhibition are assumed to have the capacity to accumulate 2-carboxyarabinitol 1-phosphate, an important inhibitor of Rubisco. This information gives further insight into the light-dependent regulation of Rubisco.
- 4. The changes in Rubisco activity through the period from pre-dawn to dusk showed differences among CO_2 treatments with increased Rubisco activity at lower CO_2 concentrations. Rubisco activity increased as light levels increased during the day similarly in all CO_2 treatments. However, Rubisco activity decreased at dusk at a faster rate in the

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subambient than ambient or superambient CO2 treatments.

Stomatal Density

- 1. Leaf stomatal density tended to increase with increasing ${\rm CO}_2$ concentration from the 160 to the 500 ppm ${\rm CO}_2$ treatment followed by a leveling off in the response among the superambient treatments.
- 2. The CO₂ treatments resulted in greater changes in stomatal density in the abaxial than adaxial leaf surfaces.

Total Nonstructural Carbohydrates

- 1. Early in the growing season, the total nonstructural carbohydrate concentration (TNC) of the rice plants increased with increasing ${\rm CO_2}$ treatment. However, by the middle of the growing season response to ${\rm CO_2}$ treatment began to level off among the superambient treatments.
- 2. As the season progressed, differences in TNC among ${\rm CO}_2$ treatments decreased and by the end of the growing season there was little difference in TNC among the ${\rm CO}_2$ treatments.
- 3. The decline with time in TNC differences among ${\rm CO}_2$ treatments was greater in the TNC of the leaves and grain than the stems (stem and leaf sheaths).
- 4. The total amount of non-structural carbohydrate in the above ground tissue increased markedly with increasing ${\rm CO}_2$ concentration and leveled off among the superambient treatments.
- 5. The TNC was very low (<1% dry weight) in root tissue.
- 6. It was found that in rice, the culm plays a major role

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in the storage of carbohydrate.

Sugars and Starch

- 1. Sucrose was found to be a major pool in the leaves and culms in rice (<15% dry weight) along with starch (also <15% dry weight). Fructose glucose and fructan concentrations were low (<1% dry weight).
- 2. Early in the season, the level of starch and sucrose increased with increasing CO₂ concentration, with starch showing the greatest response. Towards the end of the season, little change in either pool was observed.
- 3. Early in the growing season, the subambient treatments displayed little diurnal change in starch levels while starch levels greatly increased from the morning to the afternoon in the superambient treatments. Later in the season, the response was similar in all CO₂ treatments.
- 4. Early in the season, there was a significant rise in sucrose levels during the day but by later in the season, sucrose concentrations were similar at both times in the days in all the CO₂ treatments.

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Section II

Developmental Responses of Rice

to Photoperiod and

Carbon Dioxide Concentration

J.T. Baker, L.H. Allen, Jr., K.J. Boote, J.W. Jones and

P.H. Jones

II. Developmental Responses of Rice to Photoperiod and Carbon Dioxide Concentration.

ABSTRACT

The documented increase in the carbon dioxide concentration of the earth's atmosphere has stimulated interest in the effects of CO2 on plants and in particular the future prospects for the world's food supplies. While rice is a major food crop, relatively little is known about the effects of CO2 concentration on the timing of physiological growth stages and total growth duration which are important aspects of a rice cultivar's adaptability to the environment of a particular geographic region. The objectives of this study were to determine the developmental responses of a modern, improved rice cultivar (Oryza sativa, cv. IR-30) to a range of CO2 concentrations under two contrasting photoperiods. Rice plants were grown season-long in outdoor, naturally-lit, computer controlled environment, plant growth chambers in CO2 concentrations of 160, 250, (subambient) 330 (ambient), 500, 660, and 900 (superambient) μ mol CO₂ mol⁻¹ air. The entire experiment was conducted twice during 1987. The first or early planted rice (EPR) experiment was conducted with photoperiod extension lights during the vegetative phase of development while the second or late planted rice (LPR) experiment was conducted using only naturally occurring photoperiod. In both experiments, mainstem leaf developmental rates were greater during

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vegetative than reproductive growth stages and leaf appearance rates increased with CO2 treatment during vegetative development. In the LPR experiment, panicle initiation and boot stage occurred earlier and total growth duration was shortened for rice plants in the superambient compared with ambient and subambient CO2 treatments. This acceleration of plant development with increasing CO2 treatment was associated with a CO2 induced decrease in the number mainstem leaves formed during the vegetative phase growth. The reduced developmental response of rice plants to CO2 in the EPR compared with the LPR experiment attributed to the artificially extended photoperiod during the EPR experiment forcing a delay in the onset of reproductive development particularly in the superambient treat-The CO₂ induced acceleration of development and shortening of total growth duration should become a topic of interest for rice agronomists and breeders involved with selecting rice cultivars and agronomic practices for a particular geographic region in view of the continued increases in global atmospheric CO2 concentration.



INTRODUCTION

The carbon dioxide concentration of the earths atmosphere during the last ice age may have been as low as 160 to 200 μ mol CO₂ mol⁻¹ (Delmas et al. 1980). More recently, CO₂ concentration has increased from about 315 μ mol mol⁻¹ in 1958 to over 345 μ mol mol⁻¹ in 1985 (Bacastow et al., 1985) and is expected to double sometime during the next century due mainly to the continued burning of fossil fuels (Baes et al., 1976).

Carbon dioxide concentration affects many plant processes mainly through its direct effects on photosynthesis and stomatal physiology. As a result, elevated CO2 levels have been shown to increase growth and yield of C3 grasses (Cock and Yoshida, 1973; Fischer and Aguilar, 1976; Gifford, 1977; Sionit et al. 1981). However, the effects season-long exposure to various CO2 concentrations on timing of the physiological growth stages for rice has not been studied. Since rice provides half the diet of billion people and contributes between a fourth and a of the diet for another 400 million (Swaminathan, 1984) it important to determine the developmental responses of rice to a changing CO2 environment.

The development of rice consists of three main phases: vegetative, reproductive and ripening (Stansel, 1975). The vegetative phase covers the period of time from emergence to panicle initiation and is characterized by the initiation and appearance of leaves and tillers. The reproduc-

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tive phase, from panicle initiation to anthesis, is marked by the development of the panicle and cessation of leaf initiation. The ripening phase, from anthesis to maturity, involves grain filling and ripening.

Rice is generally considered to be a short day plant with panicle initiation being delayed or prevented by exposure to long days (Vergara and Chang, 1985). While there is considerable diversity among rice cultivars in photoperiod sensitivity, differences in growth duration are largely due to differences in the duration of vegetative growth. Thus, early maturing cultivars typically have a shorter vegetative growth phase than later maturing cultivars (Vergara and Chang, 1985). Growth duration and the effects on it of photoperiod and temperature largely determine the adaptability of a cultivar to a particular region (Chang and Vergara, 1971).

The objectives of this study were to determine the developmental responses of paddy-grown rice to season-long exposure to subambient, ambient and superambient levels of carbon dioxide concentration and further, to compare these responses under two contrasting photoperiods.

MATERIALS AND METHODS

Controlled Environment Chambers

Rice (Oryza Sativa L., cv. IR-30) plants were grown season long in six controlled environment chambers described in detail by Jones et al. (1984). These chambers are exposed to natural sunlight and constructed of clear acrylic tops 2.0 x 1.0 m in cross section by 1.5 m in height. The tops were attached to an aluminum vat, filled with soil and measuring 1.5 x 0.8 m in cross section and 0.5 m deep in order to provide water tight, flooded root environment for growing rice in paddy culture.

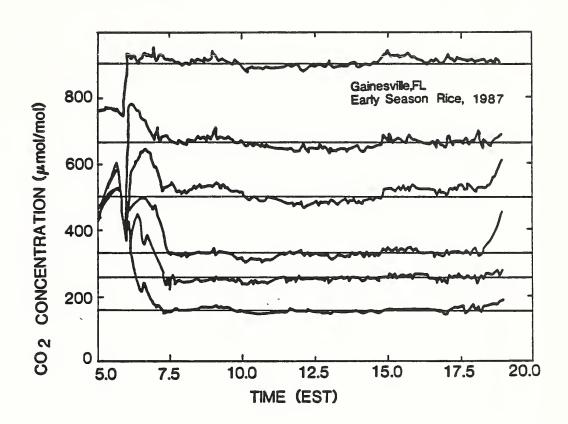
In each chamber, daytime carbon dioxide concentration was maintained at either 160, 250 (subambient CO_2 treatments), 330 (ambient CO_2 treatment), 500, 660 or 900 (superambient CO_2 treatments) $\mu\mathrm{mol}$ CO_2 mol^{-1} air (Fig. 1). A computer controlled, solenoid valve actuated, CO_2 injection system (Jones et al., 1984) was used to replace the CO_2 taken up by the plants during photosynthesis. A similar control algorithm was used to maintain subambient CO_2 levels by injecting CO_2 -free air into the subambient chambers during periods of low photosynthetic rates experienced early in the season prior to canopy closure or during periods of low light intensity.

Dry bulb and dewpoint air temperatures were controlled to 31 and 18°C, respectively. Paddy flood water depth was maintained at 5 cm above the soil surface and flood water temperature was maintained between 27 to 29°C using re-

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Fig. 1. Example of the quality of ${\rm CO_2}$ concentration control for six ${\rm CO_2}$ treatments on 6 April 1987 for the early planted rice. Horizontal lines indicate ${\rm CO_2}$ concentration target levels of 160, 250, 330, 500, 660, 900 $\mu{\rm mol}$ ${\rm CO_2}$ mol⁻¹ air. Carbon dioxide concentrations were monitored at 300 s intervals.

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sistance heaters consisting of plastic coated nichrome heating wire wrapped around aluminum rods and placed in the flood water.

Plant Culture

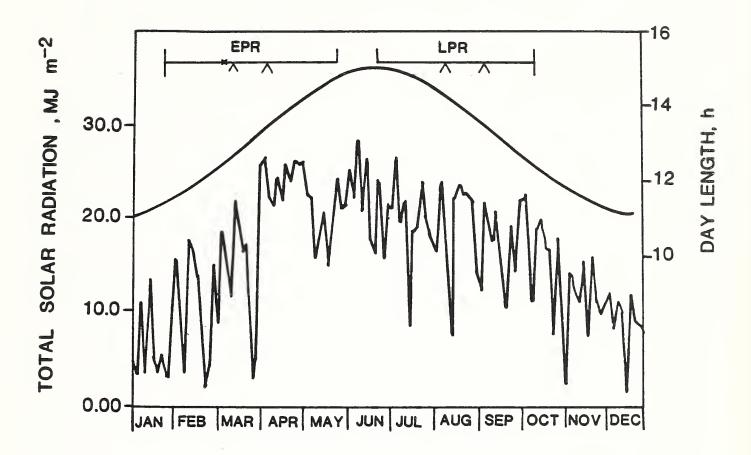
This experiment was conducted twice during 1987 with planting dates of 22 Jan. (early planted rice, EPR) and 23 June (late planted rice, LPR), respectively (Fig. 2). In order to compare the developmental responses of rice to CO₂ concentrations under different photoperiods, the photoperiod was extended during the EPR experiment with four supplemental, incandescent, 75 watt lights from 1700 to 2400 Eastern Standard Time (EST) until 2 Mar. 1987 (Fig. 2).

In both experiments, rice seeds were direct planted, by hand, into 11 rows, 0.18 m apart in each chamber. Plants were thinned to 235 plants m⁻² near the second leaf stage and the paddies were flooded on 3 Feb. and 2 July 1987 for the EPR and LPR, respectively. Shades were maintained at canopy height along the outside of each chamber in order to simulate light conditions found in a field crop.

The soils used were a Chandler fine sand (sandy, siliceous, hyperthermic, uncoated, Typic Quartzsamments) and a Zolfo fine sand (sandy, siliceous, hyperthermic, Grossarenic Entic Haplohumods) for the EPR and LPR, respectively. Just prior to the application of flood water, each chamber was fertilized with N, P, and K, at rates of 12,

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Fig. 2. Total radiation and daylength (civil twilight to civil twilight) for the EPR and LPR experiments, respectively. The $\hat{}$ symbols indicate the time of panicle initiation and anthesis for the ambient (330 μ mol mol $^{-1}$) CO₂ treatment in both experiments. The x for the EPR experiment indicates the day when photoperiod extension lights (photoperiod extended to greater than 18 h) were removed.



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11, 11 and 10, 9, 9 g m⁻² for the EPR and LPR, respectively. Additional N was applied at rates of 4.5, 4.5 and 8.0 g m⁻² at 32, 51, and 61 days after planting (DAP), respectively for the EPR and at rates of 4.8, 4.8, and 9.5 g m⁻² at 23, 42, and 64 DAP, respectively for the LPR. Fertilizer N was applied as urea in all cases.

Developmental Measurements

The main stems of five replicate plants in each chamber were tagged with a small plastic loop prior to flooding in both experiments. Stage of leaf development was determined after Haun (1973) for these tagged mainstems from laminar length measurements made 2-3 times weekly. scale growth units were calculated from these data by dividing laminar length of the last expanded leaf by the visible laminar length of the growing leaf and adding this ratio to the leaf number of the last expanded leaf. Thus, a mainstem with a leaf stage of 8.4 has eight expanded leaves with the visible laminar length of the growing ninth leaf being four-tenths of the length of the last expanded leaf. At 19 DAP for the LPR, main stem laminar length measurements and calculation of Haun scale growth units were made on 10 destructively sampled plants for each chamber.

Dates for panicle initiation were determined twice a week by dissection of mainstems from five randomly sampled plants from each chamber. The developing panicles at this point were 1 to 4 mm in length measuring from the developing neck node to the tip of the panicle. Heading and

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physiological maturity were evaluated on a canopy basis in both experiments.

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RESULTS AND DISCUSSION

Leaf Appearance

In both experiments and in all CO2 treatments, plots Haun scale growth units against days after planting revealed two distinct rates of leaf appearance: a initial rate associated with the vegetative phase of development followed by a slower rate associated with late vegetative and early reproductive development. A typical example of this shift in leaf appearance rate is shown Fig. 3 for a tagged plant in the 330 μ mol mol⁻¹ CO₂ treatment of the EPR experiment. Similar decreases appearance rate have been described for rice at panicle initiation (Yoshida, 1977; Vergara, 1980) and for spring wheat near double ridge formation (Baker et al., For all mainstems in both experiments, five leaves, including the flag leaf, appeared during this second slower phase of leaf appearance. This sudden shift in leaf appearance rate which occurred prior to panicle initiation both experiments.

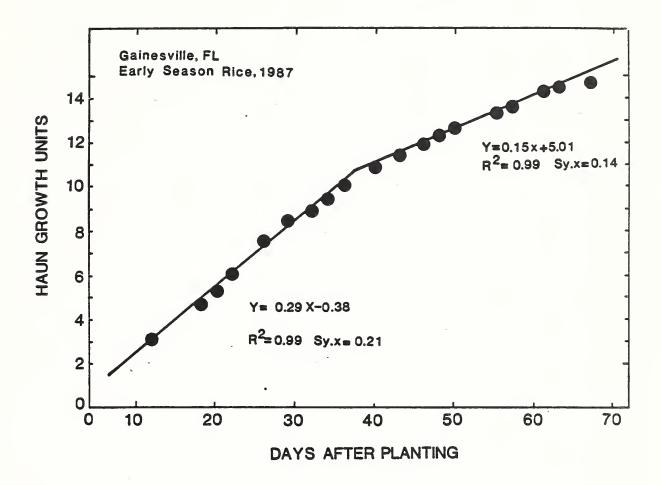
The number of days between the appearance of successive leaves, called the phyllochron interval (PI) (Klepper et al. 1982), was determined from the reciprocal of the slope of the linear regression of Haun scale growth units against DAP separately for each tagged mainstem and for the vegetative and reproductive phases of growth. The R² values for these regressions exceeded 0.97 in all cases.

The PI for the vegetative phase ranging from 3.3 to 4.1

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Fig. 3. Haun growth units vs. days after planting for a tagged plant in the 330 $\mu mol~{\rm CO_2~mol^{-1}}$ air treatment in the EPR experiment.

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days leaf-1 while that of the reproductive phase ranged from 6.4 to 9.9 days leaf $^{-1}$ across the CO_2 treatments (Table 1). Vegetative phase PI decreased from the subambient to the ambient and superambient treatments for the EPR (Table 1). This small but measurable increase in developmental rates with increasing CO2 concentration can affect both growth and yield since potential tillering rate becomes exponential after about the fourth mainstem stage (Yoshida, 1981). Although there were no measured significant differences in vegetative phase PI for the LPR plants (Table 1), the measured mainstem Haun growth stage for the 10 sampled plants at 19 DAP were : 6.5 ± 0.2 , 6.8 ± 0.3 , 6.9 ± 0.4 , 6.9 ± 0.4 , 7.2 ± 0.5 , and 7.3 ± 0.2 leaves for the 160 through the 900 μ mol CO₂ mol⁻¹ treatments, respectively, again providing evidence for a slight acceleration in leaf development with increased CO2 treatment. Gifford (1977) found no CO2 effects on PI of wheat plants grown in pots in a phytotron experiment, while Imai and Murata (1979) found small increases in leaf appearance for rice plants exposed to 1000 μ mol mol⁻¹ CO₂ treatments compared with 300 μ mol mol⁻¹ controls.

Main-stem Leaf Number

The regression equations of Haun scale growth units against DAP were used to calculate the main-stem leaf stage of the tagged plants at panicle initiation. For the LPR but not the EPR experiment, increasing CO₂ treatment resulted

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Effect of CO₂ treatment on number of days for phyllochron interval during vegetative and reproductive phases of development of early planted (EPR) and late planted rice (LPR). Table 1.

		Phyllochron interval	interval	
S	Vegetat	Vegetative pháse	Reproduc	Reproductive phase
Treatment	EPR	LPR	EPR	LPR
umol mol-1		days leaf	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	
160	4.1	3.6	7.1	8.6
250	4.1	3.7	9.9	6.6
330	3.6	3,3	6.9	8.6
200	3.4	3.8	7.0	8.7
099	3.3	3.6	6.4	7.4
006	3.6	3.6	6.7	8.0
LSD _{0.05}	9.0	NS	NS	1.3

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in a decrease in mainstem leaf number at panicle initiation leading to a reduction in final mainstem leaf number (Table 2). This decline in leaf number with increasing CO_2 treatment for the LPR was due to an apparent CO_2 induced reduction in the number of leaves formed during the vegetative phase of growth. Differences in leaf number between the two experiments are attributed to the photoperiod extension lights used in the EPR which caused a delay in the onset of reproductive development, especially in the ambient and superambient CO_2 treatments. Thus, the effects of CO_2 on rice developmental rates can be modified by photoperiod.

Reproductive Development

For tagged main stems in both experiments, increasing CO2 treatment resulted in a decrease in the number of days from planting to the day of the sudden shift in leaf appearance rate or the beginning of reproductive phase leaf development (BRPLD) (Table 3). In a similar fashion, days to panicle initiation, and boot stage (Table 3) were also decreased with increasing CO2 treatment. Similarly, Imai et al., (1985) found that flowering occurred six days earlier under CO₂ enrichment and that CO₂ enriched plants had fewer mainstem leaf than ambient controls. Differences growth stage among EPR treatments (Table 3) can be attributed to differences in vegetative phase PI (Table since final leaf number was very similar among EPR treatments (Table 2). The greater response of LPR to CO2 treatment in days to the BRPLD and panicle initiation

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Table 2. Effects of CO₂ treatment on mainstem leaf number at panicle initiation and boot stage for early planted (EPR) and late planted rice (LPR).

CO Treatment	Mainstem sta panicle	Mainstem Haun growth stage at panicle initiation EPR LPR	Final leaf EPR	Final mainstem leaf number PR LPR
-pmol mol-1-		leaves	aves	
160	12.1	12.9 NS	15.4	15.3 NS
250	12.0	12.0 NS	14.6	14.2 NS
330	11.8	11.6 NS	14.8	13.6**
200	12.1	10.7**	14.8	13.0**
099	12.2	10.5**	14.6	12.8*
006	12.0	10.5**	14.6	12.8*
L.SD _{0.05}	NS	0.8	NS	0.8

*, ** Indicates significant difference between planting date at a particular CO, level as measured by the t-test at the 0.05 and 0.01 levels of confidence, respectively.

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Table 3. Effects of ${\rm CO}_2$ treatment on developmental stages of early planted (EPR) and late planted rice (LPR).

Treatment EPR LPR - Lumol mol - 1	1	tcle in	Panicle initiation EPR LPR	Boot stage	stage
	35**			FF	LPR
	35**	after	planting	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1
	1	54	22	11	77 NS
	29 NS	54	22	71	81 NS
	26*	20	51	71	SN 69
	25**	20	45	69	99 99
	24*	20	45	99	¥69
900 34	25**	20	45	<i>L</i> 9	62 NS
LSD _{0.05} 3.6	4.5			7.1	9.7

*, ** Indicates significant difference between planting date at a particular CO₂ level as measured by the t-test at the 0.05 and 0.01 levels of confidence, respectively.

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attributed mainly to a decrease in vegetative phase leaf number (Table 2). The lack of significant difference between the two experiments in days to boot stage can be attributed to a consistent, although usually not significant, longer reproductive phase PI for the LPR than EPR (Table 1).

These results point to a decrease in growth duration with increasing CO_2 concentration. This in turn may affect a cultivar's adaptability to a particular geographical region as the global atmospheric CO_2 concentration continues to increase in the future.

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SUMMARY

In both experiments, leaves appeared more slowly tagged mainstem tillers during the late vegetative early reproductive stages of development than during early vegetative stage of growth. It is suggested that this sudden decrease in leaf appearance rate may signal a shift by the plant from vegetative to reproductive development prior to the beginning of panicle initiation. In both experiments, a slight decrease in leaf developmental rates detected during the vegetative phase of growth subambient compared with superambient CO2 treatments. In the LPR experiment, with increasing CO2 treatment, final mainstem leaf number was decreased due to the production of fewer mainstem leaves during vegetative development. resulted in a decrease in the duration of vegetative development with increasing CO2 treatment. The CO2 induced reduction in leaf number and growth duration seen the LPR was largely prevented by artificially extending photoperiod during the vegetative stage of growth.

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Section III

Growth and Yield Responses of Rice to

Subambient, Ambient, and Superambient

Carbon Dioxide Concentrations

J.T. Baker, L.H. Allen, Jr., and K.J. Boote

III. Growth and Yield Responses of Rice to Subambient, Ambient, and Superambient Carbon Dioxide Concentrations.

ABSTRACT

The current global increase in atmospheric CO2 concentration, is expected to result in a doubling of present CO2 levels expected sometime during the middle of the next This has stimulated interest in the effects of century. CO2 concentration on plants and in particular, food crops. The objectives of this study were to determine the growth and grain yield responses of rice (Oryza sativa, L., cv. IR-30) to season-long exposure to a range of CO2 concentrations under naturally sun-lit conditions. Rice plants were grown in paddy culture in outdoor, controlled-environment, plant growth chambers during two separate experiments and exposed to subambient (160 and 250), ambient (330), or superambient (500, 660, 900 μ mol CO_2 mol⁻¹ air) CO_2 treat-Total above-ground biomass, biomass of individual plant parts, tillering, and final grain yield followed a similar trend with CO2 treatment: an increase in growth and yield response across the subambient and ambient CO2 treatments to the 500 μ mol mol⁻¹ CO₂ treatment followed by a leveling off of the response across the superambient treat-Differences in biomass and laminar area among CO2 ments. treatments were largely due to corresponding differences in tillering response. Panicles plant⁻¹ was the yield component almost entirely responsible for differences in final

grain yield among CO_2 treatments. Doubling the CO_2 concentration from 330 to 660 $\mu\mathrm{mol}$ CO_2 mol^{-1} air resulted in a 38% increase in grain yield. However, a similar grain yield increase was obtained at the 500 $\mu\mathrm{mol}$ mol^{-1} CO_2 treatment. Based on this, it is concluded that the growth and grain yield increases of IR-30, in response to the current global rise in atmospheric CO_2 concentration, may be obtained long before the doubling of atmospheric CO_2 concentration expected during the middle of the next century.

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INTRODUCTION

The current global increases in atmospheric CO2 concentration are now well established (Keeling, 1983) these increases are largely attributed to the continued burning of fossel fuels and large scale deforestation (Woodwell et al., 1978). However, based on gasses trapped in ice cores from the Antarctic and Greenland, Barnola et al. (1983) have estimated that the atmospheric CO2 concentration may have been as low as 180 μ mol CO₂ mol⁻¹ air 15,000 to 20,000 years ago. Ice core studies have placed the atmospheric CO_2 concentration at around 280 μ mol mol⁻¹, by the end of the last ice age (Berner et al., 1980; Delmas et al. 1980; Neftel et al., 1982). Based on the results of Neftel et al. (1985) and findings from other ice core studies (Barnola et al., 1983; Raynaud and Barnola, 1985), Gammon and Fraser (1985) estimate the 1880 or pre-industrial CO₂ concentration at 265 to 285 μ mol CO₂ mol⁻¹ air; a range of values little changed since the end of the last Continuous measurement at Mauna Loa, Hawaii ice age. (Keeling et al., 1982) have traced the rise in atmospheric CO_2 concentration from 315 μ mol mol⁻¹ in 1958 to a current level of about 348 μ mol mol⁻¹. This increase in atmospheric CO2 concentration is expected to continue and result in a doubling of current levels sometime during the next century (Trabalka et al., 1986).

Elevated CO₂ levels have been shown to increase growth and yield of many C₃ food crops (Kimball, 1983; Cure,

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1985). However, only a few studies have dealt with the season-long effects of CO₂ concentration on small grain cereals. Rice is an extremely important food crop and is the only major cereal grain used almost exclusively for human consumption. Furthermore, rice provides half of the diet of 1,600 million people, while another 400 million people depend on rice for one-fourth to half of their diet (Swaminathan, 1984).

The increased growth of small grain cereals under CO₂ enrichment has often been associated with an increase in tillering for both wheat (Sionet et al., 1980; 1981a,b; Gifford, 1977) and rice (Imai and Murata, 1976; 1979a,b; Imai et al., 1985). As a result, CO₂ enriched plants usually produce greater yields due mainly to an increase in the number of grain bearing wheat heads (Gifford, 1977; 1979; Sionet et al., 1980; 1981a,b) or grain bearing rice panicles (Imai et al., 1985). However, increases in individual grain mass have also been reported for wheat (Sionet et al., 1980; 1981a,b) and rice (Imai et al., 1985).

The objectives of this study were to determine the growth and final grain yield responses of rice grown season long in paddy culture, under naturally sunlit conditions and exposed to subambient, ambient and superambient CO_2 concentrations. Reasons for including the subambient treatments are two-fold. First, as previously mentioned, CO_2 concentration of the earth's atmosphere was considerate

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bly lower in the past. The response of C_3 plants to subambient levels is therefore of historical as well as physiological interest. Second, the subambient treatments were included in order to provide a more complete growth and yield response surface to CO_2 concentration.

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MATERIALS AND METHODS

Controlled Environment Chambers

Rice (Oryza Sativa L., cv. IR-30) plants were grown season long in six controlled environment chambers described in detail by Jones et al. (1984). These chambers are exposed to natural sunlight and are constructed of a clear cellulose acetate roof with mylar walls. Above ground chamber dimensions are 2.0 x 1.0 m in cross section by 1.5 m in height. These chamber tops were attached to an aluminum vat, filled with soil and measuring 1.5 x 0.8 m in cross section and 0.5 m deep in order to provide a water tight, flooded root environment for growing rice in paddy culture.

In each chamber, daytime carbon dioxide concentration was maintained at either 160, 250 (subambient $\mathrm{CO_2}$ treatments), 330 (ambient $\mathrm{CO_2}$ treatment), 500, 660 or 900 (superambient $\mathrm{CO_2}$ treatments) $\mu\mathrm{mol}$ $\mathrm{CO_2/mol}$ air. A computer controlled, solenoid valve actuated, $\mathrm{CO_2}$ injection system (Jones et al., 1984) was used to replace the $\mathrm{CO_2}$ taken up by the plants during photosynthesis. A similar control algorithm was used to maintain subambient $\mathrm{CO_2}$ levels by injecting $\mathrm{CO_2}$ -free air into the subambient chambers during periods of low photosynthetic rates experienced early in the season, prior to canopy closure or during periods of low light intensity.

Dry bulb and dewpoint air temperatures were controlled to 31 and 18°C, respectively. Paddy flood water depth was

maintained at 5 cm above the soil surface using a float actuated watering valve. Flood water temperature was controlled to between 27 to 29°C using resistance heaters consisting of plastic coated nichrome heating wire wrapped around nine 0.8 m long aluminum rods and placed within the flood water.

Plant Culture

The experiment was conducted twice during 1987 with planting dates of 22 Jan., for the early planted rice (EPR) experiment and 23 June for the late planted rice (LPR) experiment. In both experiments, rice was direct seeded by hand, into 11 rows in each chamber, 17.8 cm apart. The plants were thinned to 235 plants m⁻² and flood water was applied at the second leaf stage in both growing seasons. Shades were maintained at canopy height along the outside of each chamber in order to provide a light environment found in a field crop.

Immediately prior to the application of the flood, the soil in each chamber was fertilized with N, P, and K at rates of 12, 11, 11 and 10, 9, 9 g m⁻² for the EPR and LPR, respectively. In all cases, fertilizer N was applied as urea. Additional N was applied at rates of 4.5, 4.5, and 8.0 g m⁻² at 32, 51, and 61 days after planting (DAP), respectively for the EPR and at rates of 4.8, 4.8, and 9.5 g m⁻² at 23, 42, and 64 DAP, respectively for the LPR. The soils used were a Chandler fine sand (sandy, siliceous,

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hyperthermic, uncoated, Typic Quartzsamments) and a Zolfo fine sand (sandy, siliceous, hyperthermic, Grossarenic Entic Haplohumods) for the EPR and LPR, respectively.

Growth and Yield Measurements

In the LPR experiment, plants were destructively sampled at 19, 30, 44, 58, 71, 86, and 110 DAP. to avoid the effects of a constantly declining plant population on measured growth and yield attributes, rows cent to the randomly predetermined sampling positions treated as border rows and left undisturbed through out the growing season. At 19,44, and 71 DAP, 20 plants were pled from each chamber, while at 86 DAP, 10 plants were sampled. On these sampling dates, half of the plants collected from the east side of the chamber and the remaining half from the west side of each chamber. The east and west sides of each chamber were treated as replicates in a completely randomized design. Each sampled plant was detached at ground level and the number of living leaves, tillers and panicles, when present, were counted. area per plant was measured photometrically with a area meter (LI-COR, LI-3000, Lincoln Nebraska). Severe leaf rolling of detached lamina of plants sampled at 86 prevented an accurate measure of laminar area for sampling date. Dry weights were determined separately for leaf laminae, stems including leaf sheaths, and panicles after oven drying at 70°C for 48 h.

Mathematical growth analyses were conducted after Kvet

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et al. (1971) for the replicated harvests collected at 19, 44, and 71 DAP. For these samples, calculations were made for: 1) biomass increment (AW,) the amount of above ground biomass produced between harvests, 2) net assimilation rate (NAR), amount of biomass produced per unit leaf area per unit time, and 3) leaf area duration (LAD), the amount of leaf area present during a harvest interval.

Ten and fifteen plants for the EPR and LPR, respectively, were sampled from each of three center rows for yield and yield component analyses. Growth attributes of each plant were measured as previously described and grain number and grain and chaff dry weight per panicle were determined after threshing and oven drying each individual panicle. Since the total above ground biomass, grain yield, and yield component measurements were rarely significantly different, as measured by the t-test at the 0.05 level of significance, between the EPR and LPR experiments, these data were further summarized by treating the EPR and LPR results as replicates in a completely randomized design.

In the LPR experiment, three cylindrical, 1.6 l, polyvinyl chloride pots with a rooting depth of 0.5 m were filled with soil and situated on the center of each of three rows on the floor of the chamber vats prior to filling the vats with soil. Nine plants were grown in each pot in the same row orientation with the rest of the plants

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outside the pots. Except for the pot, cultural and environmental conditions for plants inside and outside the pots were the same. At 30, 58, and 111 DAP, one pot from each chamber was removed and sampled. Growth and yield attributes of the above ground portion of each plant were determined as previously described. Each pot, with soil and roots still intact, was then frozen solid and sectioned with depth, at 5 cm depth intervals measuring from the top of the pot. For each 5 cm soil depth segment, the soil and roots were then thawed and separated. Root length for each soil layer was determined after Newman (1966) using a 1 cm grid and root biomass was determined after oven drying. Mathematical growth analyses were conducted, as previously described, after Kvet et al. (1971) for the samples collected at 30 and 58 DAP.

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RESULTS

Biomass accumulation

The temporal trends in total above-ground biomass accumulation for the LPR in each of the six ${\rm CO}_2$ treatments are shown in Fig. 1. Above-ground biomass increased with increasing ${\rm CO}_2$ treatment from the 160 to the 500 $\mu{\rm mol}$ ${\rm CO}_2$ ${\rm mol}^{-1}$ air treatment and remained relatively constant among the superambient treatments. By the end of the growing season, above-ground biomass of the superambient treatments was more than double that of either the subambient treatments (Fig 1).

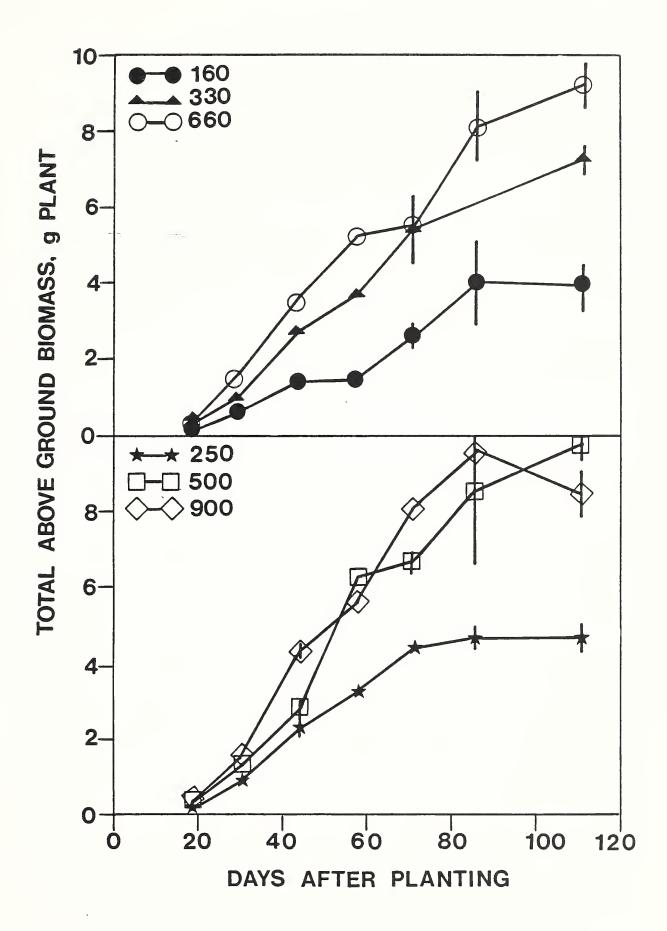
Results of previous studies on small grain crops have also shown enhanced dry matter accretion with ${\rm CO}_2$ enrichment for both rice (Imai and Murata, 1976, 1979a,b; Imai et al., 1985) and wheat (Gifford, 1977, 1979; Sionit et al., 1980, 1981a,b). However, this leveling off of the growth response among the superambient ${\rm CO}_2$ treatments, beginning at the 500 μ mol mol⁻¹ treatment, suggests that at least for the rice cultivar IR-30, under the environmental conditions defined here, the expected increased growth resulting from the current rise in global atmospheric ${\rm CO}_2$ concentration may begin to level off long before the expected doubling of ${\rm CO}_2$ levels projected to occur during the next century.

In Figs. 2-4 are the temporal trends in biomass of the above ground plant parts. Stem biomass (Fig. 2), including that of the leaf sheaths, and panicle biomass (Fig. 3), responded to the CO_2 treatments in a similar fashion as

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Fig. 1. Temporal trends in total above ground biomass for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.

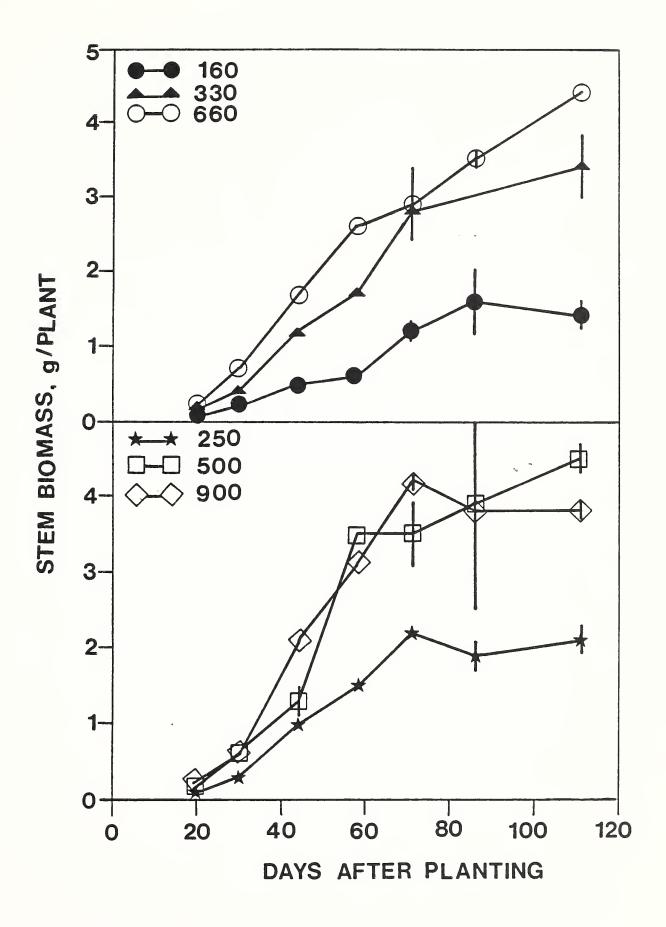
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Fig. 2 Temporal trends in stem (including leaf sheath) biomass for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.

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Fig. 3. Temporal trends in panicle biomass for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.

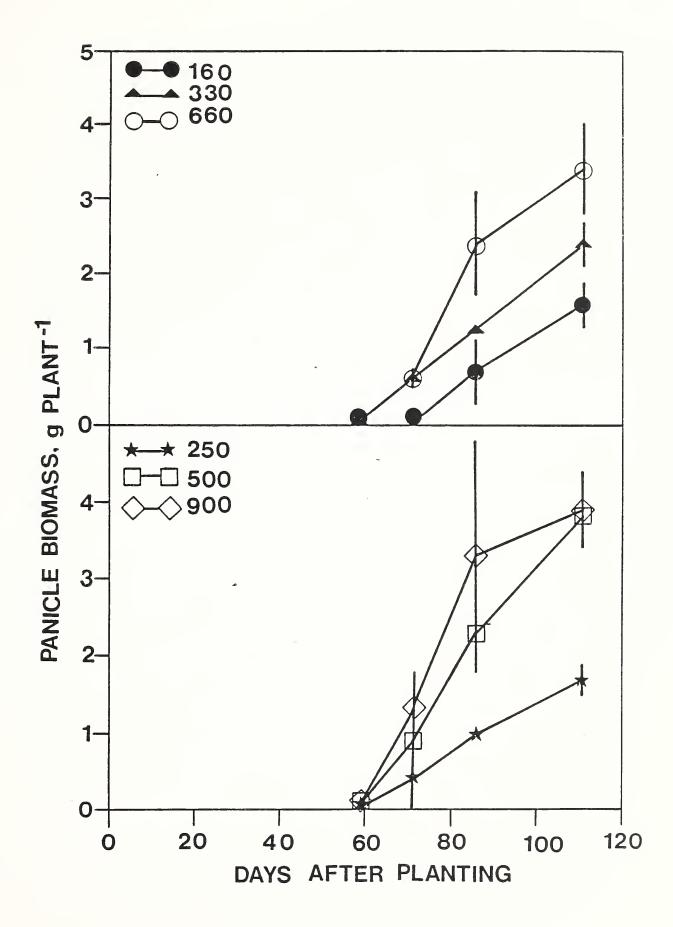
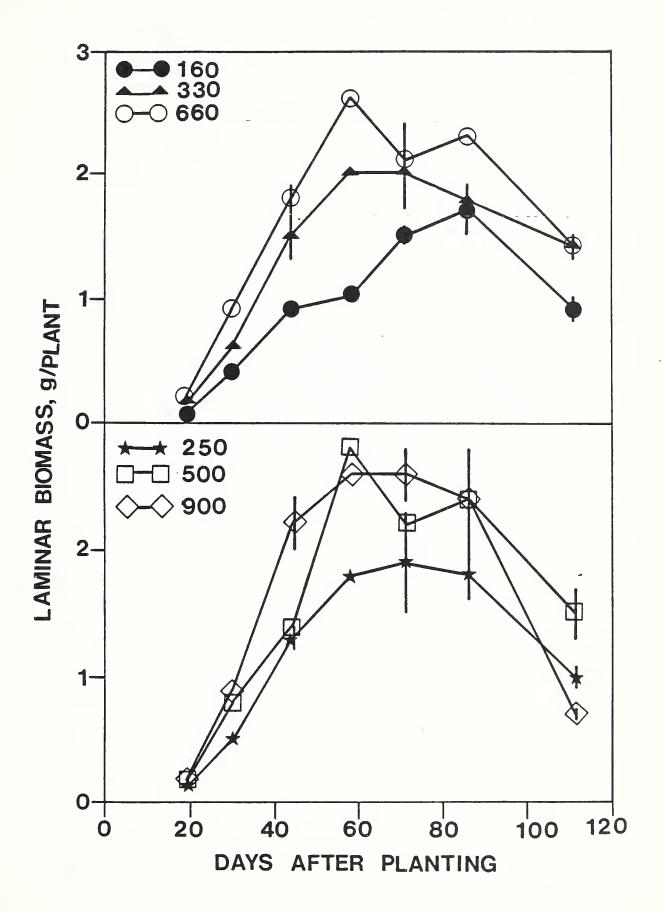


Fig. 4. Temporal trends in laminar biomass for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.



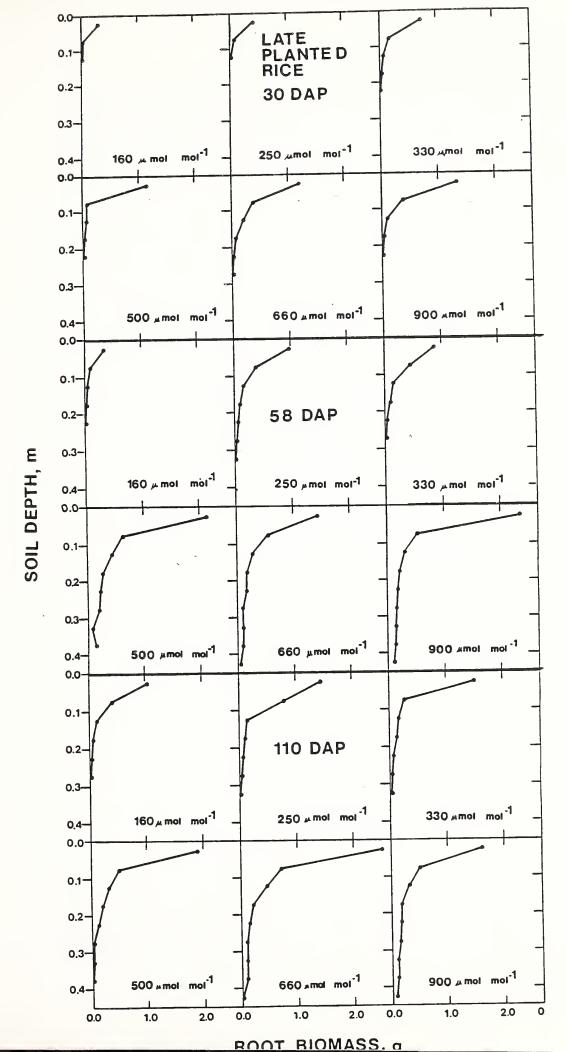
that observed for total above ground biomass (Fig. 1): an increase in stem and panicle biomass from the 160 to the 500 μ mol mol⁻¹ CO₂ treatment followed by a leveling of the growth response across the superambient treatments. The general trend in laminar biomass (Fig. 4) with CO₂ treatment was essentially the same as previously described for stem and panicle biomass. However, the magnitude of the response of laminar biomass to CO₂ treatment was not as great as that for the other plant parts including total above ground biomass.

Total above ground biomass and stem biomass increased with time during the growing season in a similar fashion (Figs. 1 and 2). Laminar biomass (Fig. 4) reached a peak and began to decline, due mainly to the senescence of older lower leaves, at about the same time the panicles began to appear and grow rapidly (Fig. 3).

Biomass accumulation by the roots (Fig. 5) followed a similar response to ${\rm CO_2}$ treatment as did the above ground portion of the plants: an increase in growth from the 160 to the 500 μ mol mol⁻¹ treatment followed by a leveling of the growth response among the superambient treatments. In addition to growing faster, roots in the superambient treatments penetrated the soil to greater depths than roots in the ambient and subambient treatments. By 58 DAP, a few of the roots in the superambient treatments had extended to the bottom most soil layers. In all treatments the greatest root biomass was found in the upper soil layers generally

Fig. 5. Root biomass vs. soil depth at 30, 58; and 110 days after planting for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations.

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above 0.2 m. By 58 DAP the rice root systems in all treatments had essentially attained their ultimate size and maximum depth of penetration (Fig. 5).

The partitioning of biomass between root and shoot was most affected by CO₂ treatment early in the growing season (Table 1). With the root:shoot ratios increasing with increasing CO₂ treatment. Increases in root:shoot ratios with CO₂ enrichment have also been reported for rice (Imai and Murata, 1976; Imai et al., 1985), wheat (Sionit et al., 1981), and other C₃ grasses (Imai and Murata, 1976; Patterson and Flint, 1980). The root:shoot ratios declined with time during the growing season (Table 1). Yoshida, (1981) also describes a similar decline in root:total plant dry weight ratio for rice, with time during the growing season, with ratios ranging from 0.2 at the seedling stage to 0.1 at heading.

The root length density determinations (Fig. 6) followed almost identically the same trends that were found for the root biomass measurements (Fig. 5), both with time during the growing season and with ${\rm CO}_2$ treatment. This finding indicates that there was little effect of ${\rm CO}_2$ treatment on average root thickness.

Laminar Area

The temporal trends in laminar area plant⁻¹ as affected by CO₂ treatment are shown in Fig. 7. Comparison of Fig. 7 with Fig. 1 indicates that CO₂ enrichment had a greater effect on plant biomass than on laminar area. This finding

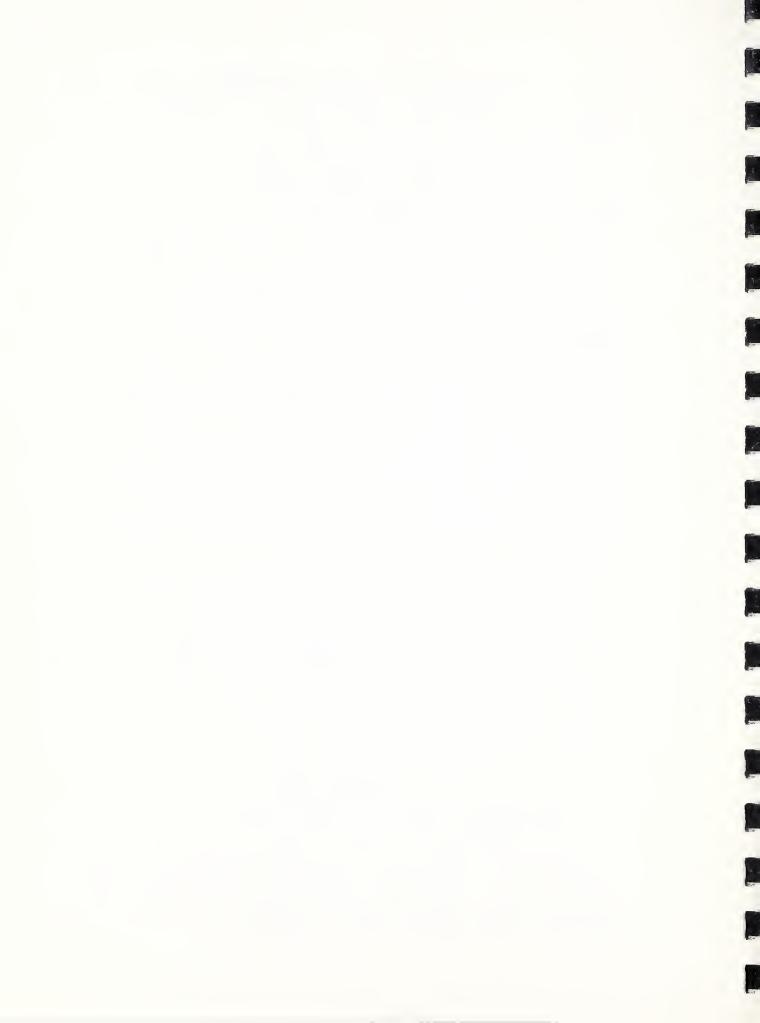


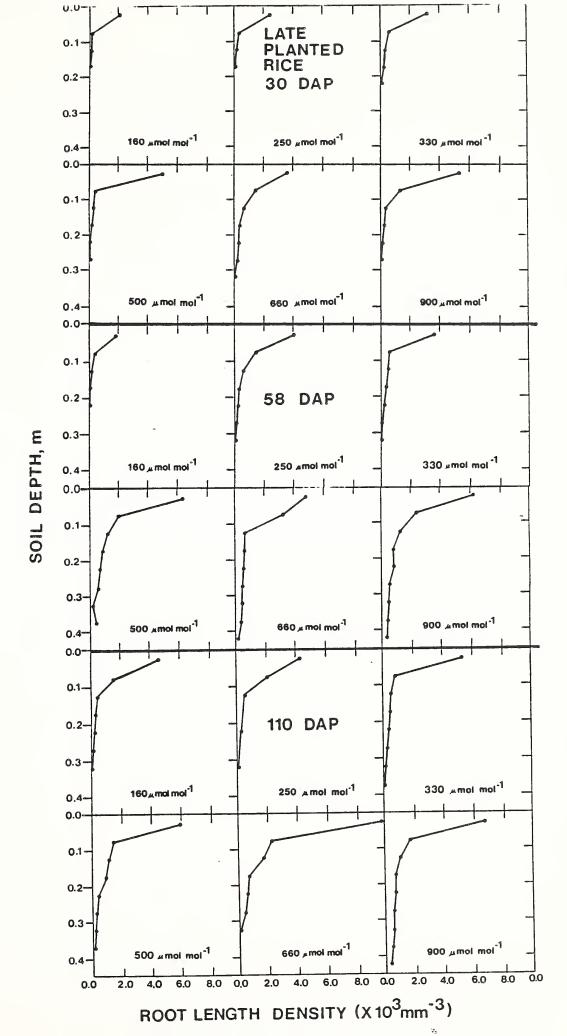
Table 1. Ratio of root biomass to total plant biomass (root:shoot ratio) at three sampling dates for the late planted rice.

		Days after plant	ing
CO ₂ Treatment	33	58	111
	- 		
-µmol mol ⁻¹ -		g/g	,
160	0.06	0.04	0.04
250	0.06	0.06	0.04
330	0.10	0.05	0.04
500	0.10	0.06	0.07
660	0.12	0.06	0.07
900	0.12	0.07	0.05
900	0.12	0.07	0.05

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Fig. 6. Root length density vs. soil depth at 30, 58, and 110 days after planting for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations.

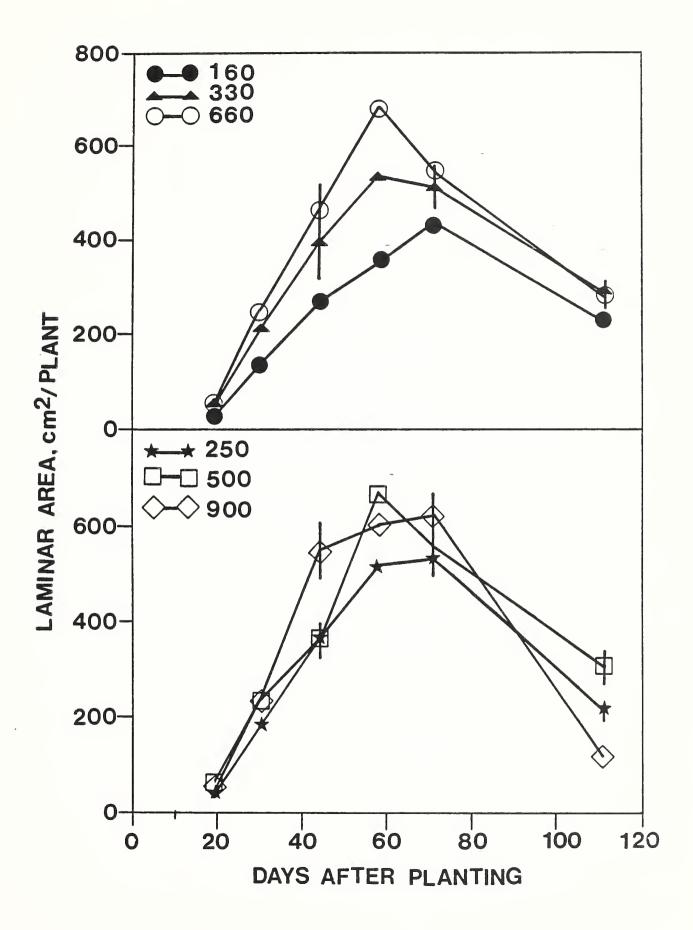
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Fig. 7. Temporal trends in laminar area for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.

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is consistent with the results of Imai and Murata (1976) and Imai et al. (1985) who also found that CO_2 enrichment of rice resulted in a relatively greater biomass than laminar area response. In order to more fully explore this differential response of biomass and laminar area to CO_2 enrichment, laminar plant⁻¹ was separated into its component parts using the following equation:

$$LAPP = TNO * LPT * LAPL$$
 [1]

where LAPP is laminar area plant⁻¹, TNO are the number of tillers plant⁻¹, LPT is the average number of leaves tiller⁻¹, and LAPL is the average laminar area leaf⁻¹. In this way, differences in laminar area among CO₂ treatments can be explained in terms of differences in one or more of these three components.

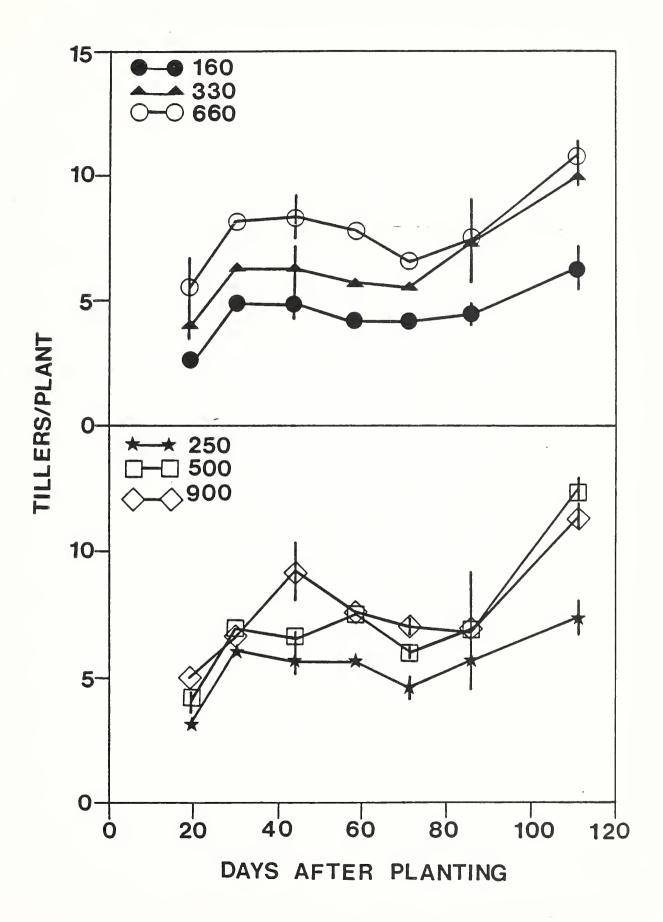
The tillering response to CO_2 treatment (Fig. 8) was very similar to that previously determined for biomass accumulation (Fig. 1): an increase in tillering with CO_2 treatment from the 160 to the 500 $\mu\mathrm{mol}$ mol^{-1} treatment followed by very little further response across the superambient treatments. Previous studies have also found increases in biomass and leaf area associated with increases in tillering under CO_2 enrichment for both wheat (Gifford, 1977; Sionit et al. 1981a,b) and rice (Imai and Murata, 1979a,b; Imai et al. 1985).

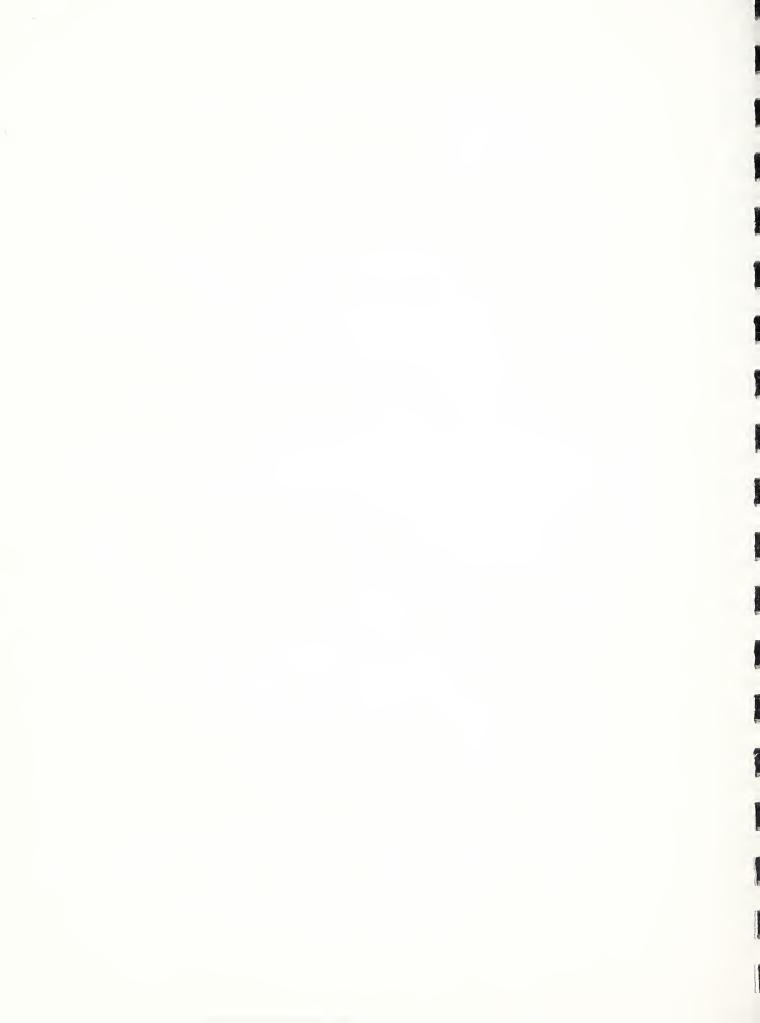
In all CO₂ treatments, rice plants reached a maximum in tiller number by 30 DAP (Fig. 8). This maximum was

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Fig. 8. Temporal trends in tillers/plant for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO}_2$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE. of two measurements. Vertical bars represent \pm SE.

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followed by a decline in tiller number as some of the weaker, later formed, tillers were aborted during stem extension and heading. This rise and subsequent decline in tiller number is a typical growth pattern for small grain cereals. The CO₂ treatments appeared to affect the magnitude but not the overall pattern of tiller production and abortion.

The rise in tiller number at the end of the growing season (Fig. 8) was due to the production of small, fective tillers during and following seed fill. The relatively greater end-of-season tillering response of the superambient compared with subambient CO2 treatments suggests a greater ratooning ability of the CO2 enriched plants. This is not unexpected since auxillary bud development is largely controlled by the internal carbohydrate status of the plant (Langer, 1963; Jewiss, 1972; Simons, 1982). In the LPR experiment, total nonstructural carbohydrate concentration of the plants in the superambient treatments was greater than that of the plants in the subambient and ambient CO2 treatments throughout the growing season (Section VII). Furthermore, tillering is suppressed by environmental factors which result in decreased internal plant carbohydrate concentrations such as light intensity (Friend, 1965; Willey and Holliday, 1971; Williams et al., 1975) or high temperatures, especially high nighttime temperatures (Langer, 1963; Owen, 1971).

The seasonal trends in the second and third components

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of leaf area, LAPL and LPT, are shown in Figs. 9 and 10, respectively. The LAPL, increased with time due to the production of successively larger leaves on each tiller up to the second or third leaf below the flag leaf and the senescence of smaller and older lower leaves. Except for the 160 CO₂ treatment in which LAPL was occasionally lower than that of the other treatments, LAPL was little affected by CO₂ treatment. In contrast, CO₂ enrichment in other plant species has been shown to increase leaf area by increasing both the size and number of leaves (Mauney et al., 1978; Hardy and Havelka, 1977; Morison and Gifford, 1984). In this experiment, differences in laminar area among the CO₂ treatment can be attributed almost entirely to differences in the number of leaves plant⁻¹ (Fig. 11) rather than differences in leaf size (Fig. 9).

There was a strong tendency, especially in the mid portion of the season, for LPT to decrease with increasing CO_2 concentration. Differences in LPT typically ranged from about 0.5 to one leaf among the CO_2 treatments (Fig. 9). This decrease in LPT with increasing CO_2 treatment partially offset the effects of increased tillering (Fig. 8) and leaf number (Fig. 11) on laminar area with increasing CO_2 treatment.

Mathematical Analyses of Growth

Shown in Table 2 are the results of the mathematical

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Fig. 9. Temporal trends in laminar area/leaf for rice plants grown to maturity in subambient, ambient and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE..ls 2

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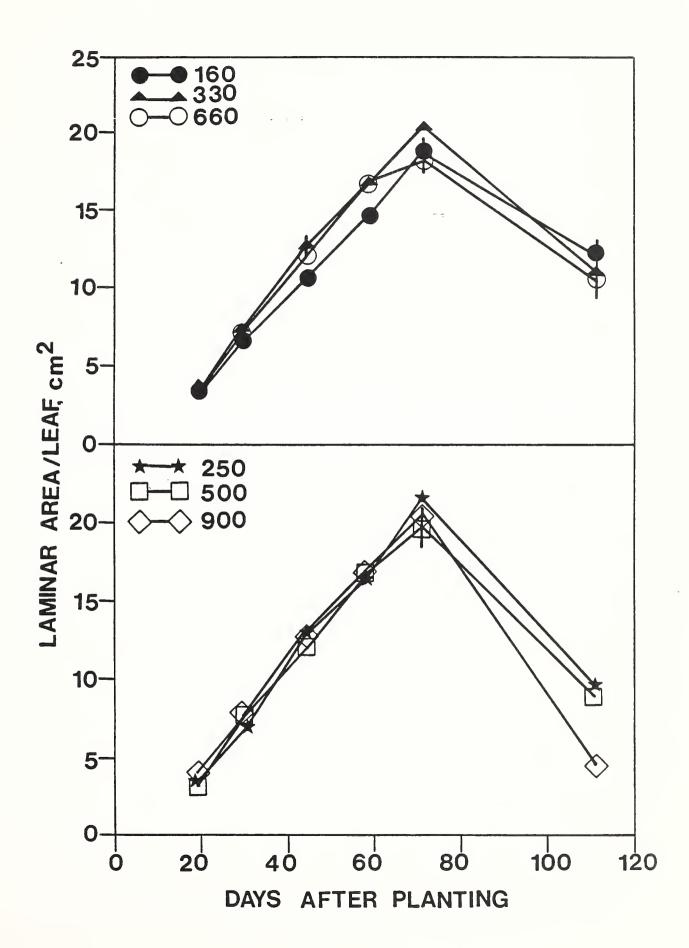


Fig. 10. Temporal trends in the average number of leaves/tiller for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.

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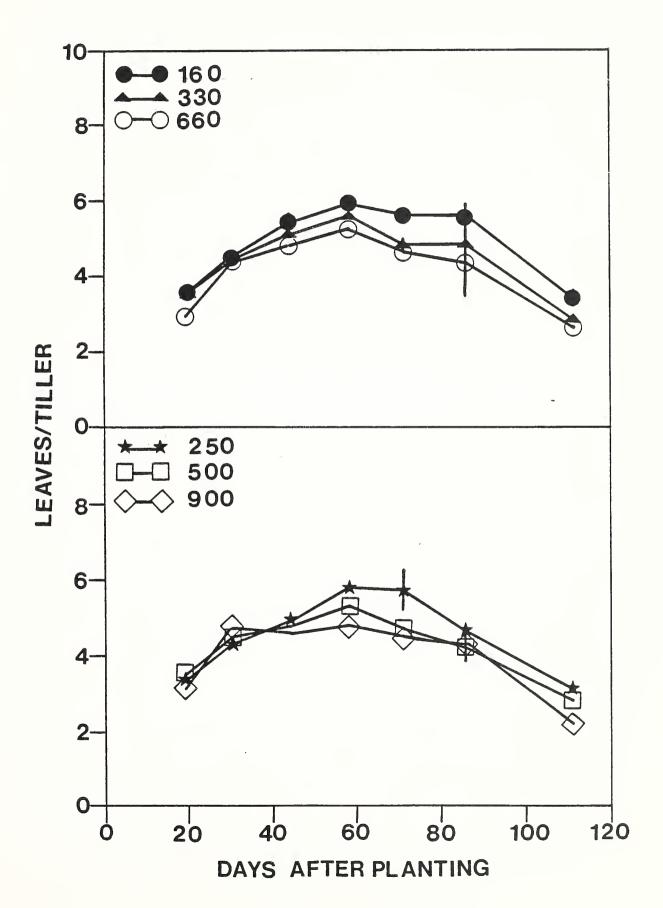
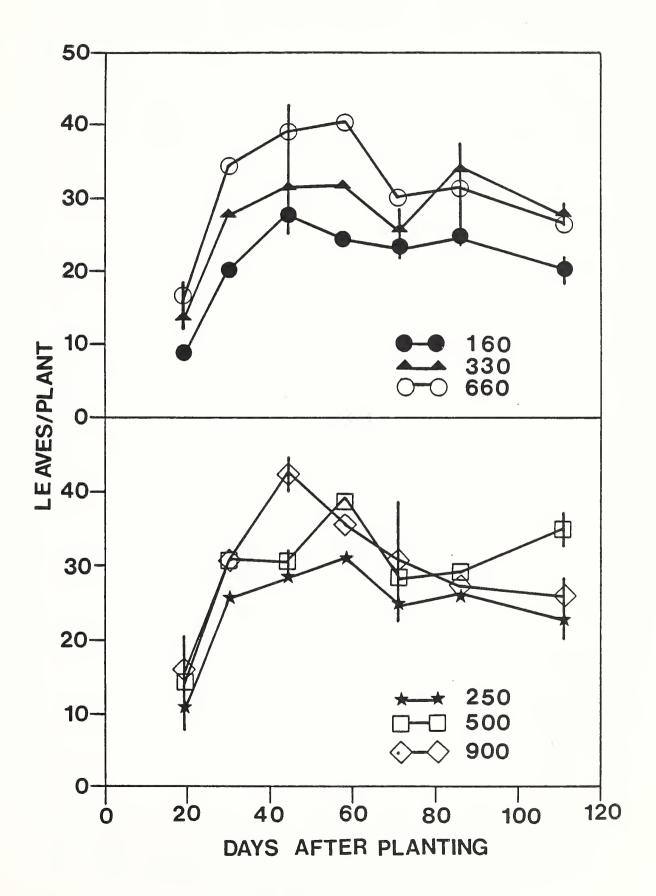


Fig. 11. Temporal trends in leaves/plant for rice plants grown to maturity in subambient, ambient and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.

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Effect of CO, enrichment on biomass increment (ΔW), net assimilation rate (NAR), and leaf area duration (LAD) (± S.E.M.) for late planted rice grown to maturity in controlled environment chambers during three harvest intervals. Table 2.

Treatment	MΔ	NAR	LAD	ΜV	NAR	LAD	ΜV	NAR	LAD
pmol mol-1	6	p _Z _w 6	m ² d	9.	g m ⁻² d	m ² d	6	g m ⁻² d	m ² d
	Har (19-44 days,	Harvest interval I (19-44 days, root biomass not included)	I not included)	Harv (30-58 days,	Harvest interval II (30-58 days, root biomass included)	II included)	Harv (44-71 days	Harvest interval III (44-71 days, root biomass not included)	III not included)
160	1.2 ± 0.0	4.9 ± 0.2	0.37 ± 0.02	6.0	1.5	0.64	1.3 ± 0.3	1.4 ± 0.4	0.96 ± 0.01
250	2.1 ± 0.2	6.0 ± 0.3	0.50 + 0.04	2.5	2.9	0.89	2.2 ± 0.3	1.8 ± 0.4	1.20 ± 0.04
330	2.5 ± 0.1	6.2 ± 0.9	0.55 + 0.10	2.8	2.9	0.97	2.7 ± 0.8	2.3 ± 0.3	1.23 ± 0.17
200	2.5 ± 0.3	6.0 + 9.9	0.51 ± 0.01	5.4	4.6	1.16	4.0 ± 0.1	3.3 + 0.3	1.24 + 0.09
099	3.2 ± 0.1	6.9 ± 0.3	0.65 ± 0.05	3.8	3.1	1.21	2.0 + 0.6	1.4 ± 0.1	1.46 ± 0.06
006	3.9 ± 0.1	7.2 ± 0.5	0.76 + 0.08	4.4	4.0	1.09	3.8 ± 0.1	2.4 ± 0.1	1.58 ± 0.02

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growth analyses for three harvest periods. Since biomass increment (AW) is roughly equivalent to the product of NAR and LAD (Patterson et al., 1979), differences in AW among CO₂ treatments can be explained in terms of these two components.

The (^{A}W) increased with CO_2 treatment and was relatively similar across the three harvest intervals for most of the CO_2 treatments. In general, NAR and LAD increased with increasing CO_2 treatment, while NAR decreased and LAD with time during the growing season (Table 2). Both ^{A}W and NAR have been shown to increase with CO_2 enrichment for both rice (Imai and Murata, 1979a,b) and wheat (Sionit et al., 1981a).

The second harvest interval (30-58 days, Table 2) was included in order to compare the effect of including root biomass on the calculations of these growth parameters. The seasonal trends in calculations of NAR and LAD were apparently little affected by the root biomass as evidenced by the relatively continuous trends in NAR and LAD across these three harvest intervals. This result is not unexpected since root biomass represented a small and constantly decreasing fraction of total plant biomass (Table 1).

With increasing ${\rm CO}_2$ treatment, specific leaf area (SLA) decreased across the two subambient ${\rm CO}_2$ treatments with little difference in SLA among the ambient or superam-



bient treatments (Fig. 12). Similarly, Sionit et al. (1981a) found no difference in SLA for wheat with CO₂ enrichment. Gifford (1977) found no difference in wheat specific leaf weight in subambient, ambient or superambient treatments. Differences in SLA may arise through differences in leaf thickness and/or differences in photoassimilate accumulation (Patterson and Flint, 1980) such as starch or sugar (Hofstra and Hesketh, 1975) or differences in structural and protein dry matter per unit leaf area (Allen et al., 1988). Patterson and Flint (1980) attributed increases in specific leaf weight for soybean and velvetleaf in CO₂ enrichment treatments to increased starch accumulation.

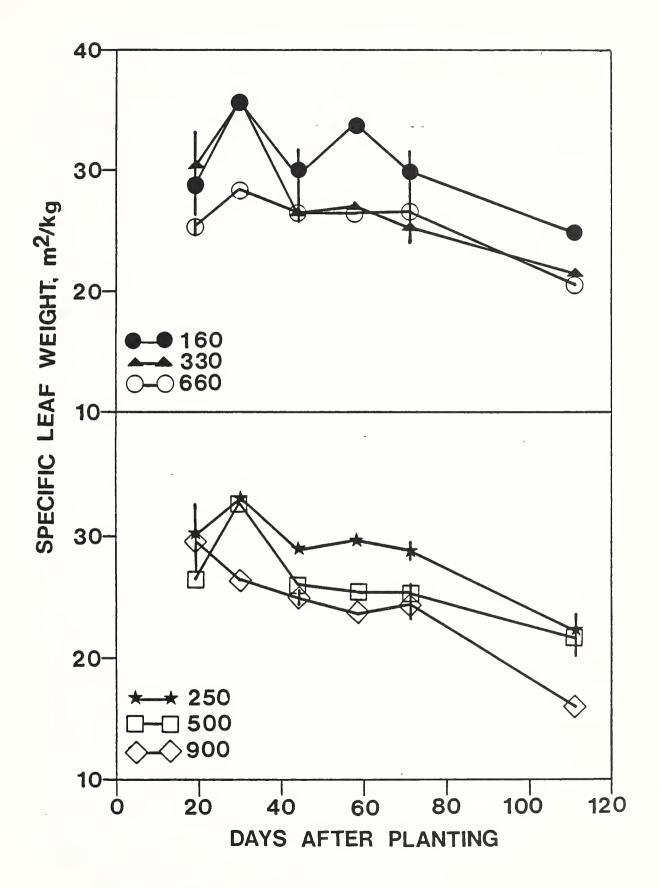
Grain Yield

Grain yield, yield components, total above ground biomass, and harvest index for both the EPR and LPR are shown in Table 3. These measurements of growth and yield attributes were very similar between the two growing seasons and differed significantly in only two of these measurements.

Final grain yield followed a similar pattern of response to CO_2 treatment, as was previously described for biomass (Figs. 1 and 5) and tillering (Fig 8): an increase in grain yield with increasing CO_2 treatment from the 160 to the 500 μ mol mol⁻¹ treatments and a leveling off of the response across the superambient treatments. Examination of the yield components (Table 3) shows that the number of

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Fig. 12. Temporal trends in specific leaf weight for rice plants grown to maturity in subambient, ambient, and superambient ${\rm CO_2}$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE..ls 2



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Grain yield, components of yield, total above ground biomass and harvest index (+ S.E.M) for early planted (EPR) and late planted rice (LPR) grown to maturity in controlled environment chambers. Table 3.

5		Grain vield			Panicles		•	Filled grain			Grain mass	
Treatment	EPR	LPR	Avg.	EPR	EPR LPR Avg.	Avg.	EPR	LPR	Avg.	EPR	LPR	Avg.
umol mol-1		Mg ha ⁻¹			no. plant ⁻¹		0u	no. panicle -1	1	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	g 1000 ⁻¹	
160	3.6 ± 0.3	3.6 ± 0.3 3.1 ± 0.5	3.4ct	3.5 ± 0.4	3.5 ± 0.4 3.6 ± 0.5	3,6c	27 ± 3.7	27 ± 3.7 22 ± 0.8	24.84	16.5 ± 1.6 17.4 ± 0.2	17.4 ± 0.2	17.0a
250	5.1 ± 0.2	3.0 + 0.5	4.1c	5.0 + 0.4	5.0 ± 0.4 4.6 ± 0.4	4.8bc	25 ± 2.3 17 ± 4.2	17 + 4.2	20.8a	18.1 ± 0.3	18.1 ± 0.3 18.2 ± 0.3	18.2a
330	5.2 + 0.2	4.2 ± 0.6	4.8bc	5.9 ± 0.6	5.9 ± 0.6 5.5 ± 0.2	5.7ab	23 ± 2.4 19 ± 1.9	19 + 1.9	21.0a	17.1+ 0.4	17.9 ± 0.4	17.6a
200	*	6.8 ± 0.3		1	7.3 ± 0.6		8 9 8	23 ± 2.7		•	18.1 ± 0.2	
099	6.8 ± 1.0	6.3 + 1.3	6.6ab	6.9 ± 0.2	6.0 ± 0.1	6.5ab	6.5ab 25 ± 3.4 25 ± 5.0	25 ± 5.0	25.0a	17.2 ± 0.3	17.2 ± 0.3 18.4 ± 0.2	17.8a
006	7.1 ± 0.4	7.4 ± 1.0	7.3a	8.4 ± 0.3	8.4 ± 0.3 6.4 ± 0.1	7.49	7.4a 21 ± 2.0 28 ± 4.0	28 ± 4.0	24.8a	17.4 ± 0.1	17.4 ± 0.1 18.1 ± 0.2	17.8a

† Values followed by the same letter in each column are not significantly different by Duncan's multiple range test (P = 0.05).

Plants in 500 µmol mol treatment of the EPR experienced hydrogen sulfide toxicity in the root zone which inhibited seed-fill and reduced grain yield to levels below those measured in subambient treatments. These data were removed from subsequent analyses.

* Indicates significant difference between planting dates as determined by the t-test at the 0.5 level of confidence.

Table 3. (cont.) Grain yield, components of yield, total above ground biomass and harvest index (+ S.E.M.) for early planted (EPR) and late planted rice (LPR) grown to maturity in controlled environment chambers.

CO ₂		e ground biom			Harvest Index	
Treatment	EPR	LPR	Avg.	EPR	LPR	Avg.
µmol mol ⁻¹		g plant ⁻¹			g/g	
160	4.1 + 0.2	3.9 ± 0.6	4.0c	0.37 ± 0.01	0.34 ± 0.01	0.36a
250	5.6 ± 0.2	4.7 <u>+</u> 0.4	5.1bc	0.40 ± 0.03	0.27 ± 0.04	0.34a
330	5.5 ± 0.9	7.2 ± 0.5	6.3ab	0.42 ± 0.05	0.25 ± 0.02	0.34a
500		9.8 ± 0.7		₩ ₩ ₩	0.30 ± 0.02	
660	7.5 ± 0.4	9.2 ± 0.6	8.4a	0.40 ± 0.04	0.29 ± 0.04	0.35a
900	8.0 <u>+</u> 1.3	8.5 <u>+</u> 1.0	8.2a	0.39 ± 0.02	0.38 ± 0.03	0.39a

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panicles plant $^{-1}$ was the yield component almost entirely responsible for the observed differences in grain yield among the CO_2 treatments. In fact an almost 1:1 relationship existed between one panicle plant $^{-1}$ and one Mg ha $^{-1}$ of grain yield (Table 3) among the CO_2 treatments in both the EPR and LPR experiments.

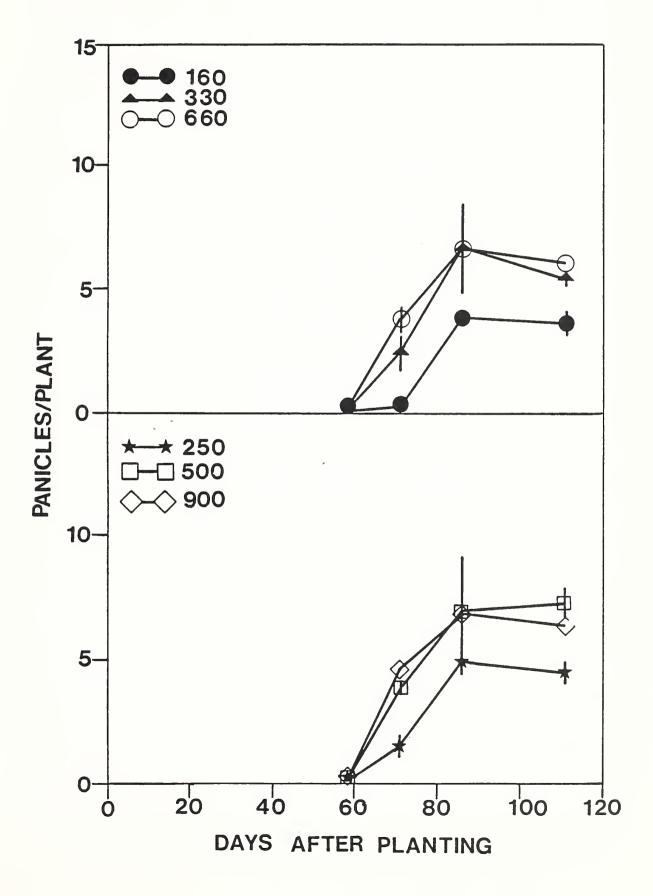
In both experiments, filled grain number panicle⁻¹ was the most variable of the yield components, while individual seed mass was very stable among CO₂ treatments. Neither of these components were significantly affected by the CO₂ treatments. Thus, in both experiments, grain yields were the result of the number of grains plant⁻¹ which in turn depended on the number of grain bearing panicles. The temporal trends in the number of panicles plant⁻¹ for the LPR are shown in Fig. 13.

In wheat, increases in the number of grain bearing heads are usually the yield component most responsible for increased grain yield under ${\rm CO_2}$ enrichment (Gifford, 1977; 1979; Sionit et al., 1980; 1981a,b). However, Sionit et al. (1980, 1981a,b) and Goudriaan and de Ruiter (1983) also reported significant increases in individual seed mass under ${\rm CO_2}$ enrichment while Gifford (1977; 1979) reported no significant ${\rm CO_2}$ effects on individual seed mass.

For rice, Imai et al. (1985) found small but significant increases in individual grain mass under ${\rm CO_2}$ enrichment. However, they attributed the majority of the yield

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Fig. 13. Temporal trends in panicles/plant for rice plants grown to maturity in subambient, ambient and superambient ${\rm CO}_2$ concentrations. Each value represents the mean of two measurements. Vertical bars represent \pm SE.



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response to CO₂ enrichment to an increase in the number of seed plant⁻¹ which resulted from an increase in the number of panicles plant⁻¹. Individual seed mass has been shown to be a relatively stable cultivar characteristic for a rice (Owen, 1969; Yoshida et al., 1972) because the growing grain is enclosed by the inner and outer glumes and cannot grow to a size greater than that permitted by the hull. The size of the hull is determined as early as 5 days before flowering (Murata and Matsushima, 1978).

Averaged across both experiments, the 160 μ mol CO₂ mol⁻¹ air treatment resulted in a 41% reduction in grain yield compared with the 330 μ mol mol⁻¹ treatment. Similarly, Gifford 1977 obtained a 43% reduction at a similar subambient CO2 level compared with an ambient control. A doubling of the CO₂ concentration from 330 to 660 μ mol CO₂ mol⁻¹ air resulted in an average grain yield increase of 38% which is in the range reported by Kimball (1983) for other C3 plant species. However, as with the biomass response (Figs. 1 and 5), the results from the LPR experiment indicate that a similar yield increase is also obtained at 500 μ mol CO₂ mol⁻¹ air. Furthermore, photosynthetic rates (Section III) were nearly identical among the superambient treatments in both experiments. These results indicate that, at least for IR-30, rice grain yield increases resulting from the current rise in atmospheric CO2 concentration will occur long before the middle of the next century when the doubling of the atmospheric CO2 concentra-

tion is expected (Edmonds et al., 1984).

Also shown in Table 3 are the final above ground biomass and calculated harvest index (HI) for both the EPR and LPR experiments. The HI of the EPR was very stable across CO₂ treatments due to roughly proportional changes in both biomass and grain yield among the CO₂ treatments. The HI of the LPR was generally lower, although usually not significantly so, than that of the EPR and much more variable among the CO₂ treatments. In wheat, CO₂ enrichment resulted in small (Goudriaan and de Ruiter, 1983) or non-significant (Sionit et al., 1980) increases in HI. For rice Imai et al. (1985) reported an increase in HI from 0.39 to 0.45 from a doubling of ambient CO₂ levels.

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SECTION IV

Rice Photosynthesis and Evapotranspiration

Responses to

Subambient, Ambient, and Superambient
Carbon Dioxide Concentrations

J.T. Baker, L.H. Allen, Jr., K.J. Boote, J.W. Jones and

P.H. Jones

IV. Rice Photosynthesis and Evapotranspiration Responses to Subambient, Ambient, and Superambient Carbon Dioxide Concentrations.

ABSTRACT

The current global rise in atmospheric CO2 concentration has stimulated interest in the effects of CO2 concentration on global vegetation and agricultural crops. A major focus in this area is the direct effects of concentration on the gas exchange processes of plants. objectives of this investigation were to determine effects of a wide range of CO2 concentrations on photosynthesis, evapotranspiration, and water use efficiency for rice (Oryza sativa, L., cv. IR-30). Rice plants were grown season-long, during two separate growing seasons in naturally sun-lit, controlled-environment plant growth chambers and exposed to subambient (160 and 250), ambient (330), or superambient (500, 660, and 900 μ mol CO₂ mol⁻¹ air) CO₂ treatments. Prior to stem extension, daily curves of photosynthetic rate vs. photosynthetic photon flux density were analyzed in order to obtain estimates of canopy light utilization efficiency (α) and canopy conductance to CO₂ transfer (τ). Estimates of α increased with increasing CO₂ treatment with the greatest increase occurring across 160 to the 500 μ mol mol⁻¹ CO₂ treatments. Estimates of τ were far more variable than those for α and were rarely significantly different among CO2 treatments. After the beginning of stem extension, the canopy photosynthetic

light response became essentially linear and showed little tendency towards light saturation. Photosynthetic rates increased with increasing CO_2 treatment from the 160 to the 500 $\mu\mathrm{mol}$ mol⁻¹ treatments followed by a leveling off of the response among the superambient CO_2 treatments. Short term cross-switching of CO_2 concentrations among the chambers revealed a profound adaptive response to CO_2 acclimation treatment. Photosynthetic rate, measured at a common external CO_2 concentration, decreased with increasing CO_2 acclimation treatment. Evapotranspiration decreased while water-use efficiency increased with increasing CO_2 concentration.

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INTRODUCTION

The current global increase in atmospheric carbon dioxide concentration has been traced by continuous measurement (Keeling et al., 1982) and is expected to result in approximate doubling of current levels sometime during the next century (Baes et al., 1977). The primary direct effect of elevated CO2 on plants with the C3 carbon fixation pathway is usually an increase in net photosynthesis (Akita and Tanaka, 1973). However, studies of short-term photosynthetic response to CO2 enrichment ignore possible long-term adaptive responses and are thus inadequate for predicting photosynthesis during long-term exposure to a particular CO2 concentration (Acock et al., 1985). The objectives of this investigation were to determine for rice: (i) the photosynthetic and evapotranspiration responses to season-long exposure to CO2 concentrations ranging from subambient to superambient levels and (ii) investigate possible long-term adaptive responses to these CO2 concentrations.

MATERIALS AND METHODS

Controlled-Environment Chambers

Rice (Oryza Sativa L., cv. IR-30) plants were grown season long in six controlled environment chambers described in detail by Jones et al. (1984). These chambers are exposed to natural sunlight and constructed of a clear cellulose acetate roof with mylar walls. Above ground chamber dimensions are 2.0 x 1.0 m in cross section by 1.5 m in height. The chamber tops were attached to an aluminum vat, filled with soil and measuring 1.5 x 0.8 m in cross section and 0.5 m deep in order to provide a water tight, flooded root environment for growing rice in paddy culture.

A dedicated computer operating in real time was used to maintain environmental control in each of the chambers and record plant response and environmental data to magnetic disc every 300 s. Signals from an array of sensors in each chamber were monitored at specific time intervals and used in a variety of feedback control algorithms to actuate various devices in order to maintain desired environmental control. Dewpoint temperature (General Eastern model 1100 DP dewpoint hygrometer) was controlled to 18°C by driving a bypass valve which determined the rate of flow of chilled water through cooling coils. The condensate from the cooling coils passed through tipping bucket rain gauges monitored by the computer to calculate evapotranspiration rate (ET). Dry bulb air temperature was controlled to 31°C by turning on and off electrical resistance heaters.

Daytime carbon dioxide concentration was maintained at either 160, 250 (subambient CO2 treatments), 330 (ambient CO2 treatment), 500, 660 or 900 (superambient CO2 treatments) μ mol CO₂/mol air using the computer controlled, solenoid valve actuated, CO2 injection system described by Jones et al. (1984). Carbon dioxide was injected from high pressure cylinders in order to replace the CO2 taken up by the plants during photosynthesis. A similar control algorithm was used to maintain subambient CO2 levels by injecting CO2-free air into the subambient chambers during periods of low photosynthetic rates experienced early in 'the season prior to canopy closure or during periods of light intensity. Details concerning the variability in environmental controls about desired setpoints for dry bulb air temperature, dew point air temperature, and CO2 concentration for these chambers are given by Jones et al. (1984, 1985a,b). Paddy flood water depth was maintained at 5 cm above the soil surface using a float actuated watering Flood water temperature was maintained between 27 and 29°C using resistance heaters consisting of plastic coated nichrome heating wire wrapped around nine 0.8 m long aluminum rods and placed between the rows within the flood water.

Canopy net photosynthetic rate (P_n) was calculated from CO_2 mass balances over each 300 s interval while photosynthetic photon flux density was integrated over the

same time interval. Water-use efficiency was calculated from these data as the ratio of $P_{\rm n}$ to ET.

Plant Culture

The experiment was conducted twice during 1987 with planting dates of 22 Jan., for the early planted rice (EPR) experiment and 23 June for the late planted rice (LPR) experiment. In both experiments, rice was direct seeded by hand, into 11 rows in each chamber, 17.8 cm apart. The plants were thinned to 235 plants m⁻² and flood water applied at the second leaf stage in both growing seasons. Shades were maintained at canopy height along the outside of each chamber in order to provide a light environment found in a field crop. Immediately prior to the application of the flood, the soil in each chamber was fertilized with N, P, and K at rates of 12, 11, 11 and 10, 9, 9 g m^{-2} for the EPR and LPR, respectively. In all cases, fertilizer N was applied as urea. Additional N was applied at rates of 4.5, 4.5, and 8.0 g m^{-2} at 32, 51, and 61 days after planting (DAP), respectively for the EPR and at rates of 4.8, 4.8, and 9.5 g m^{-2} at 23, 42, and 64 DAP, respectively for the LPR. The soils used were a Chandler fine sand (sandy, siliceous, hyperthermic, uncoated, Quartzsamments) and a Zolfo fine sand (sandy, siliceous, hyperthermic, Grossarenic Entic Haplohumods) for the EPR and LPR, respectively.

Analyses of Photosynthetic Light Response

In order to compare estimates of canopy light utilization efficiency (α) and canopy conductance to ${\rm CO_2}$ transfer (τ) among ${\rm CO_2}$ treatments, the photosynthetic light response data were analyzed by the methods outlined by Acock et. al. (1971, 1985). Canopy gross photosynthesis (${\rm P_g}$) was calculated as:

$$P_{q} = P_{n} + R_{d}$$
 [1]

where dark respiration (R_d) was averaged over a 5 h dark period (0:00 to 05:00 hrs.) from the previous night period. The P_g vs. photosynthetic photon flux density (I) were fit to a rectangular hyperbola of the form:

$$P_{q} = \alpha I \tau C / (\alpha I + \tau C)$$
 [2]

where α = canopy light utilization efficiency, τ = canopy conductance to CO_2 transfer, and C = external CO_2 concentration. Equation [2] was fit using the Gauss-Newton nonlinear least squares iterative regression method of the NLIN procedure provided by the SAS Institute.

Short-term CO2 Cross-switching Experiment

In order to evaluate the adaptive responses of the plant canopies to the ${\rm CO_2}$ treatment to which they were acclimated, a short term ${\rm CO_2}$ cross-switching experiment was conducted near boot stage from 62 to 67 days after planting (DAP) for the LPR experiment. During the morning from 7:00 to 12:00 hrs., the ${\rm CO_2}$ concentration in each chamber was controlled to 160, 330, or 660 μ mol mol⁻¹ on 62, 63, 64

DAP, respectively. In this way, canopy photosynthetic and evapotranspiration rates among the CO₂ acclimation treatments could be compared at a common external CO₂ concentration. On each day, after 12:00 hrs, the CO₂ concentration control setpoint was returned to its original acclimation treatment. This procedure was again repeated at 65, 66, and 67 DAP in order to provide additional verification of these results.

RESULTS

Treatment and Growth Stage Effects

On days with a limited range of I, such as overcast or very cloudy days, fitting eq. [2] becomes difficult and results in large errors associated with both estimates of α and τ (Acock et al., 1985). Therefore, data from days when photosynthetic photon flux density failed to reach 1300 μ mol (photons) m⁻² s⁻¹ were removed from further consideration. The EPR experiment was characterized by unusually overcast conditions throughout most of the season and therefore, few days from the EPR experiment were selected for analysis.

In Fig. 1 is an example of a P_g vs I curve fit for the 330 μ mol mol⁻¹ CO₂ treatment at 41 DAP for the EPR experiment with data points included in order to show a typical amount of scatter in the data. Comparison of P_g light response curves among CO₂ treatments (Fig. 2) revealed a pattern of response found in both experiments: an increase in photosynthetic response with increasing CO₂ concentration from the 160 to the 500 μ mol mol⁻¹ treatment with a leveling of the response among the superambient treatments.

After panicle initiation and the beginning of stem extension, the photosynthetic light response became essentially linear and showed little tendency towards light saturation (Fig. 3) in either experiment. Attempts to fit eq. [2] for canopies during or after the beginning of stem extension resulted in either a failure to converge by the

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Fig. 1. Canopy gross photosynthetic rate (Pg) vs. photon flux density at 41 days after planting for the 330 $\mu \rm mol~CO_2$ mol⁻¹ air concentration in the early planted rice (EPR) experiment.

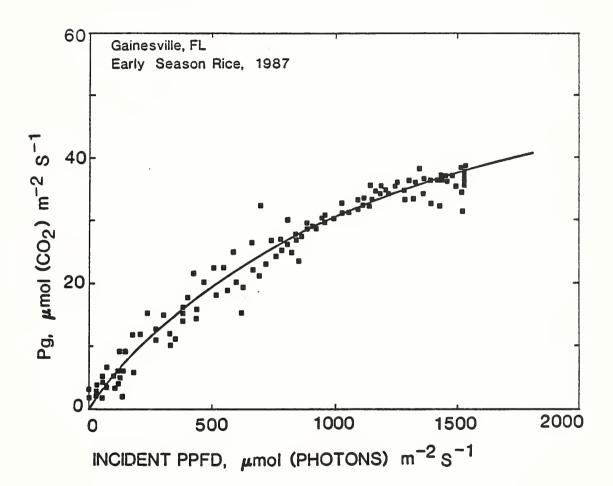


Fig. 2. Comparison of canopy gross photosynthetic rate (P_g) vs. photon flux density among the subambient, ambient and superambient carbon dioxide concentrations at 41 days after planting in the early planted rice (EPR) experiment.

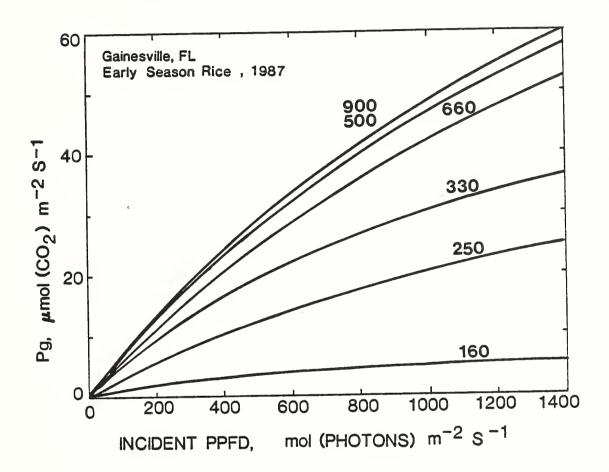
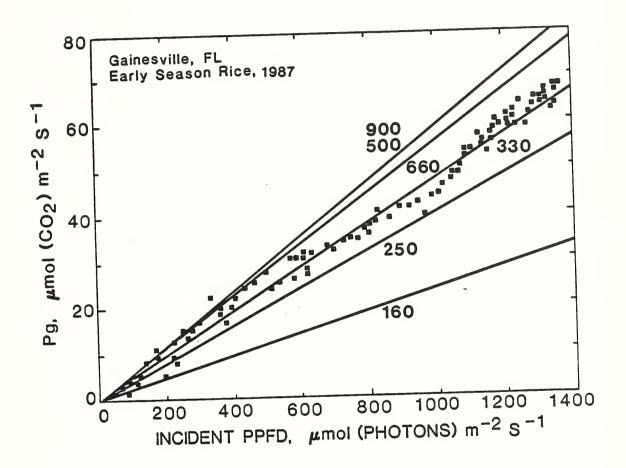


Fig. 3. Comparison of canopy gross photosynthetic rate (P_g) vs. photon flux density among the subambient, ambient and superambient carbon dioxide concentrations at 74 days after planting in the early planted rice (EPR) experiment.



SAS NLIN iterative regression technique or in the estimation of unrealistically large values of τ . Therefore, straight lines were fit to the P_n light response data collected after the beginning of stem extension using least squares linear regression. These linear regressions described the data quite well with R^2 values rarely falling below 0.90. Comparisons of photosynthetic light response among CO_2 treatments were made by comparing P_n rather than P_g in order to avoid any confounding effects of errors associated with the independent estimates of R_d .

Estimates of α and T

Values of the parameters estimated by eq. [2] during the vegetative phase of growth are shown in Table 1. Estimates of α increased with increasing CO_2 treatment with the largest increases occurring across the 160 to the 500 μ mol mol⁻¹ treatments. The errors associated with estimates of τ were much greater than those for α (Table 1) and resulted in few significant differences for τ among CO_2 treatments. Relatively greater errors associated with estimates of τ compared with α and thus difficulties in predicting τ over a growing season were also encountered by Acock et al (1985) for soybean. However, there appeared to be a trend of decreasing τ with increasing CO_2 concentration across the ambient and superambient CO_2 treatments (Table 1).

Table 1. Canopy light utilization efficiency (α) and canopy conductance to CO₂ transferred (τ) for rice grown in subambient, ambient, and superambient CO₂ treatments.

Date	Days after planting	CO ₂ concentration	α	τ
		µmol mol ^{−1} mo	ol CO ₂ mol ⁻¹ photons	mms ⁻¹
		Early plant	ed rice	
4 Mar. 1	987	160	0.011 ± 0.002a [†]	1.1 + 0.1a
		250	0.011 + 0.002a	4.8 ± 0.4b
		330	0.059 ± 0.002b	4.2 ± 0.2b
		500	0.061 <u>+</u> 0.002b	5.9 ± 0.4b
		660	0.069 <u>+</u> 0.002b	4.2 ± 0.3b
		900	0.071 <u>+</u> 0.003b	3.4 ± 0.2b
		Late planted	d rice	
12 July	1987 19	160	0.028 ± 0.002a	0.1 ± 0.1a
		250	0.032 ± 0.003ab	1.6 ± 0.1ab
		330	0.030 ± 0.002ab	3.6 ± 0.3bc
		500	0.051 <u>+</u> 0.001bc	4.2 ± 0.2c
		660	0.050 ± 0.001abc	4.1 ± 0.3c
		900	0.064 <u>+</u> 0.003c	2.8 ± 0.1abc
19 July	1987 26	160	0.041 + 0.001a	3.0 ± 0.1a
		250	0.051 + 0.001ab	6.4 + 0.3a
		330	0.052 <u>+</u> 0.003abc	10.6 ± 1.9a
		500	0.071 ± 0.001cd	8.1 ± 0.4a
		660	0.064 ± 0.002bcd	8.2 <u>+</u> 0.8a
		900	0.076 ± 0.002d	3.9 ± 0.1a

Table 1. Continued.

Date		ys after lanting	CO ₂ concentration	n a	τ
			µmol mol ⁻¹	mol CO_2 mol ⁻¹ photons	mms -1
21 July	1987	28	160	0.048 <u>+</u> 0.002a	3.9 <u>+</u> 0.1a
			250	0.045 ± 0.001ab	12.0 <u>+</u> 1.1a
			330	0.057 ± 0.001 abc	13.4 ± 1.0a
			500	0.062 ± 0.001 bc	22.7 ± 3.2a
			660	0.059 <u>+</u> 0.002abc	
			900	0.074 <u>+</u> 0.002c	11.4 ± 1.4a
22 July	1987	29	160	0.039 <u>+</u> 0.001a	5.6 <u>+</u> 0.2a
		-	250	0.042 ± 0.002a	17.3 ± 2.5a
			330	0.057 + 0.001ab	14.0 ± 0.9a
			500	0.063 ± 0.003b	20.5 ± 5.0a
			660	0.064 <u>+</u> 0.002b	24.6 ± 6.1a
			900	0.071 <u>+</u> 0.002b	14.3 ± 0.2a
26 July	1987	33	160	0.042 <u>+</u> 0.001a	7.5 <u>+</u> 0.2a
			250	0.059 + 0.002ab	12.3 ± 0.8a
			330	0.068 + 0.002bc	12.0 ± 0.6a
			500	0.080 + 0.002c	11.0 ± 0.7a
			660	0.079 <u>+</u> 0.002c	7.9 <u>+</u> 0.5a
			900	0.071 <u>+</u> 0.003c	3.4 + 0.2a

	$(\mathbb{F})_{i_1}$	

Table 1. Continued.

	after ntin <u>g</u>	CO ₂ concentratio	n ^a	τ
		µmol mol ⁻¹	mol CO ₂ mol ⁻¹ photons	mms -1
987	35	160	0.058 <u>+</u> 0.004a	6.8 <u>+</u> 0.3a
		250	0.063 ± 0.004a	13.1 ± 1.6a
		330	0.069 <u>+</u> 0.002a	10.9 ± 0.7a
		500	0.076 <u>+</u> 0.002a	10.0 ± 0.6a
		660	0.074 ± 0.002a	10.2 ± 1.0a
		900	0.076 ± 0.018a	9.2 ± 0.9a
	p1 ar		µmol mol ⁻¹ 987 35 160 250 330 500 660	pranting concent action mol CO ₂ mol ⁻¹ photons 0.058 ± 0.004a 250

 $[\]mbox{\dag}$ Parameter values for a given day, followed by the same letter are not significantly different by LSD (P < 0.05).

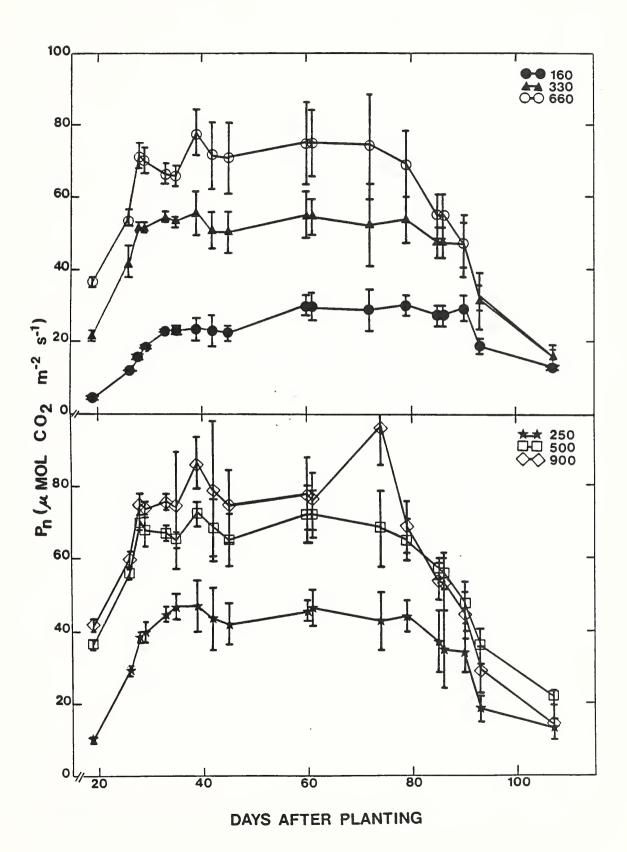


Seasonal Effects on Pn

Temporal trends in P_n at a photon flux density of 1500 μ mol (photons) m^{-2} s⁻¹ are shown in Fig. 4 for all six CO_2 treatments. Throughout the growing season, P_n increased with CO_2 treatment with the largest increases occurring from the 160 to the 500 μ mol mol⁻¹ CO_2 treatments. Differences in P_n among the superambient treatments were small and had essentially disappeared by 80 DAP.

Canopy P_n reached a maximum at about the time of panicle initiation for all CO2 treatments which corresponded with full canopy closure (Sections II and III of this This maximum rate remained relatively constant for all CO2 treatments across the growing season and only began to decline after flowering and the onset of canopy leaf senescence. The ambient and superambient treatments reached a maximum P_n earlier in the growing season than did the subambient treatments (Fig. 4). These differences in the overall pattern of P_n are similar to corresponding differences in developmental rates among CO2 treatments. Increasing CO2 treatment shortened total growth duration, and hastened development. Panicle initiation and flowering occurred 10 to 12 days earlier for the superambient compared with the 160 μ mol mol⁻¹ CO₂ treatment (Section II) in the LPR experiment. The decline in P_n at the end of the growing season also began earlier in the superambient than subambient treatments (Fig. 4).

Fig. 4. Seasonal variation in canopy net photosynthetic rate (P_n) at a photon flux density of 1500 μ mol (photons) m⁻² s⁻¹ for the subambient, ambient, and superambient carbon dioxide concentrations during the late planted rice (LPR) experiment. The points were estimated from regression equations fit to each day's data. The vertical bars represent 95% confidence intervals.



Adaptation of Pn and ET to CO2 Treatment

Shown in Table 2 are estimates of Pn calculated from regression equations using a photosynthetic photon flux density of 1500 μ mol (photons) m⁻² s⁻¹ for the short-term ${\rm CO_2}$ cross-switching experiment. Canopy P_n decreased with increasing ${\rm CO_2}$ acclimation treatment at each of the short-term ${\rm CO_2}$ switches. The magnitude of this decrease in Pn across the ${\rm CO_2}$ acclimation treatments also decreased as the ${\rm CO_2}$ concentration of the short-term switch increased. This same pattern was observed in both the 62-64 DAP and 65-67 DAP short-term ${\rm CO_2}$ switches (Table 2).

Also shown in Table 2 are hourly averages of ET at measured values of photosynthetic photon flux density near the 1500 $\mu \rm mol$ (Photons) $\rm m^{-2}$ s⁻¹ level used to compare Pn among CO₂ treatments. Measurements of ET were more variable than those for P_n at a particular light level. Values of ET tended to be similar among CO₂ acclimation treatments when measured at a common CO₂ level during the AM CO₂ cross-switch. After returning the CO₂ concentration back to the original acclimation treatment (PM switch) ET measurements tended to decrease with increasing CO₂ treatment (Table 2).

Evapotranspiration and Water-use Efficiency

In Fig. 5 is an example of the diurnal trend in ET for the 160 and 900 μ mol mol⁻¹ CO₂ treatments at 74 DAP for the EPR experiment. As expected, in both experiments, the diurnal trend in ET tracked the diurnal trend in photosyn-

estimates of Pn were calculated from regression equations of Pn vs incident PPFD with PPFD set to 1500 pmol (Photons) m s . Values of ET and PPFD represent hourly averages from 11:00 to 12:00 and 13:00 to 14:00 hrs for the AM and PM periods, respectivey. Canopy net photosynthetic rates (Pn) + one-half 95% confidence interval and evapotranspiration (ET) for rice during a short-term CO₂ concentration change over study. Estimates labeled 'AM' are from CO₂ concentrations switched to 160, 330 or 660 µmol mol prior to 12:00 h in all six chambers. Estimates labeled 'PM' are after 12:00 h when each chamber was returned to its original control setpoint. All Table 2.

Photosynthetic photon flux density AM PM	μ mol (photons) $m^{-2}s^{-1}$	1398 1402		-				1380 1418				30	
	mmol H ₂ 0 m ⁻² s ⁻¹	21.3	22.7	20.5	18.9	19.2	17.5	19.7	20.6	15,3	16.0	17.6	16.7
Evapotranspiration AM PM	mmol H ₂ (18.0	18.8	17.5	18.5	17.9	17.3	16.6	18.0	17.3	17.3	16.4	15.5
thesis PM	m ⁻² s-1	27.6 ± 2.1	43.6 ± 5.0	52.4 ± 4.0	73.6 ± 4.9	74.9 + 7.7	79.0 ± 6.4	25.2 ± 2.1	40.2 + 4.6	51.4 ± 5.9	65.6 ± 5.7	78.6 ± 15.2	79.5 ± 15.9
Net Photosynthesis AM	µmol CO ₂ m ⁻² s ⁻¹	28.0 ± 1.3	25.9 + 1.4	22.9 ± 1.3	20.6 ± 1.6	15.1 ± 0.7	9.2 + 1.3	60.4 + 4.6	55.7 ± 4.7	53.1 ± 4.7	54.1 + 4.8	48.3 ± 6.0	38.8 ± 5.2
AM CO_concentration	umol mol-1	160						330					
CO acclimation treatment	umol mol-1	160	250	330	200	099	006	160	250	330	200	099	006
Days after planting		62						63					

		,

Table 2. Continued

Photosynthetic photon flux density AM PM	µmol (photons) m ⁻² s ⁻¹	1463 1113						1467 1586					
spiration PM	mmol H ₂ 0 m ⁻² s ⁻¹	20.6	21.3	18.8	17.3	17.8	16.3	20.1	20.7	21.1	18.5	18.3	16.9
Evapotranspiration AM PM	mmol H ₂ (17.7	18.2	18.2	17.4	17.3	17.8	16.9	17.6	17.6	16.8	17.9	17.3
thesis	m-2-1	31.2 ± 3.3	46.0 ± 5.4	54.2 ± 5.2	74.9 ± 10.7	69.4 + 6.8	75.0 ± 5.9	27.7 ± 2.4	40.9 ± 5.5	49.6 + 4.8	64.1 ± 5.4	68.5 + 7.6	72.3 ± 8.2
Net Photosynthesis AM	µmol CO ₂ m ⁻² s ⁻¹	70.1 ± 1.8	66.3 ± 1.9	61.5 ± 1.6	58.0 ± 2.6	49.2 + 7.1	43.4 + 1.8	87.1 ± 5.7	86.9 ± 5.9	82.8 ± 5.1	81.0 ± 5.1	78.4 ± 5.5	69.0 ± 4.7
AM CO ₂ concentration	punol mol-1	330						099	`				
CO acclimation treatment	umol mol-1	160	250	330	200	099	006	160	250	330	200	099	006
Days after planting		99						29					

levels of PPFD during the PM period at 64 days after t Equipment failure prevented ET measurements at high planting.

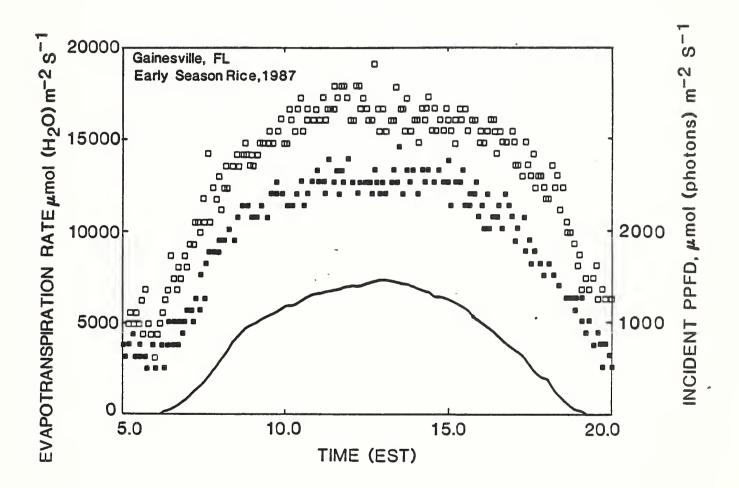
Table 2. continued

Photosynthetic photon flux density AM PM	µmol (photons) m ⁻² s ⁻¹	1473						1296 1335					
Evapotranspiration AM PM	mmol H ₂ 0 m ⁻² s ⁻¹	+						21.5	22.1	20.8	16.1	18.5	16.7
Evapotra AM	fomm	16.4	17.5	16.9	16.3	16.8	17.2	17.5	18.4	17.1	15.6	17.3	16.2
thesis	m-2 _s -1	24.4 ± 2.4	35.0 ± 6.4	48.7 ± 5.2	58.8 ± 4.8	61.4 ± 7.2	66.4 ± 6.8	28.6 ± 6.1	44.1 + 8.0	52.9 + 8.6	68.9 ± 9.1	73.4 ± 12.0	79.1 ± 8.0
Net Photosynthesis AM	µmol CO ₂ m ⁻² s ⁻¹	78.8 ± 3.4	81.6 ± 3.0	77.7 ± 2.9	79.7 ± 4.5	72.1 ± 4.0	63.2 ± 3.3	27.6 ± 2.0	24.0 ± 2.1	22.6 ± 2.3	19.5 + 1.9	14.2 ± 1.6	9.0 ± 1.4
AM CO concentration	µmol mol-1	099						160					
CO ₂ acclimation treatment	umol mol-1	160	250	330	200	099	006	160	250	330	200	099	006
Days after planting		64						99		•			

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Fig. 5. Diurnal trends in photon flux density (solid line) and evapotranspiration rates for the 160 (open symbol) and 900 (closed symbol) μ mol CO₂ mol⁻¹ air treatments at 74 days after planting in the early planted rice (EPR) experiment.





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thetic photon flux density. Comparisons of daily totals of P_n , ET, and water-use efficiency for two representative days from both the EPR and LPR experiments are presented in Table 3. As with P_n measured at high light levels (Fig. 4), daily totals of P_n increased with increasing CO_2 treatment from the 160 to the 500 μ mol mol⁻¹ CO_2 treatment followed by a leveling off in the response among the superambient treatments. Daily totals of ET tended to decrease with increasing CO_2 (Table 3). These trends in P_n and ET resulted in an increase in water-use efficiency with increasing CO_2 treatment (Table 3).

A comparison of total daily responses of rice canopies acclimated to subambient, ambient, and superambient ${\sf CO}_2$ concentrations. Table 3.

daily Total daily Water-use uptake H ₂ O loss efficiency	$_{2}$)m ⁻² mol(H ₂ 0)m ⁻² m mol (CO ₂) mol (H ₂ 0)	417.9 598.8 548.2 585.3	554.9 768.9 793.7 639.5 632.6	741.0 732.4 694.5 633.8	588.8 608.5 643.4 611.0 553.6
Total daily CO ₂ uptake	mol(CO ₂)m ⁻²	0.22 0.84 0.84 1.00	1.52 1.52 1.20 1.46 1.75		0.74 0.74 1.13 1.34 1.80
Total daily photons	mol(Photon)m ⁻²	36.0 0	. 34.3	Late planted rice	39.4
CO treatment	umol mol-1	160 250 330 500	900 160 330 500 660	160 250 330 500 660	900 160 250 330 500
Days after planting		41	74	45	61
Q		1987	1987	1987	1987
Date		4 Mar.	6 Apr. 1987	7 Aug.	23 Aug.

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DISCUSSION

Comparison of canopy gas exchange responses among season-long CO2 treatments, is complicated by the fact that CO2 enrichment can result in greater leaf area (Jones et al., 1985b; Baker et al., 1989). Jones et al. (1985b) found that soybean canopies enriched to 800 μ mol produced a maximum leaf area index (LAI) of almost twice that of the ambient controls. Rice, on the other hand, is far less responsive than soybean to CO2 enrichment effects leaf area. While CO2 enrichment results in large differences in biomass accumulation in rice, leaf area affected to a far smaller degree (Imai and Murata, 1976; Imai et al., 1985; Section III of this report). In the LPR experiment, maximum leaf area index, measured shortly after the CO2 cross-switching experiment on day 72 was significantly reduced only for the 160 μ mol mol⁻¹ treatment. On this day estimated LAI was 10.1 for the 160 μ mol mol⁻¹ treatment and ranged from 11.8 to 14.3 among the remaining CO2 treatments (Section III).

Estimates of canopy conductance to CO_2 transfer among CO_2 treatments can also be influenced by differences in canopy leaf area. However, canopy leaf area was virtually identical among the superambient treatments over the time period during which τ was estimated (Section III). The trend of decreasing τ with increasing CO_2 level among the superambient treatments is expected if the increase in CO_2

treatment results in increases in stomatal resistance or decreases the concentration and/or activity of RuBP carboxylase (Acock et al., 1985).

The range of values of α in Table 1 are similar to those reported for other C_3 plant leaves and canopies (Acock et al., 1985; Charles-Edwards, 1981; Ehleringer and Bjorkman, 1977; Jones et al., 1984). These studies have also demonstrated similar increases in α with increasing CO_2 treatment as those in Table 1. As noted by Acock et al., (1985) long term adaptation to elevated CO_2 concentration results in a far greater increase in α than can be explained by a simple corresponding difference in the carboxylating and oxygenating activity of RuBP carboxylase (eg. a decrease in photorespiration rate with increasing CO_2 treatment). The CO_2 cross-switching experiment provides further evidence of this long-term CO_2 adaptation response for rice.

Canopy ET, measured at high light levels, during the AM CO_2 cross-switch was similar among all six CO_2 acclimation treatments (Table 2). Furthermore, canopy ET measured during the AM cross-switch treatments varied little from day to day despite the large corresponding differences in the AM CO_2 concentration. The similarity in ET rates among the AM CO_2 switches appear to indicate very little differential stomatal adjustment among CO_2 acclimation treatments in response to the short term CO_2 cross-switch treatments. In contrast, the ET rates measured after re-

turning all six chambers to their original ${\rm CO}_2$ acclimation treatment tended to follow a similar trend as shown in Table 3: a decrease in ET with increasing ${\rm CO}_2$ acclimation treatment. Despite the similarity in ET during the AM ${\rm CO}_2$ switches, there was still an approximately three-fold decrease in canopy ${\rm P}_{\rm n}$ from the 160 to the 900 ${\rm CO}_2$ acclimation treatments on days 62 and 65 when the AM ${\rm CO}_2$ concentration was switched to 160 $\mu{\rm mol}$ mol⁻¹. These results suggest a profound adaptation to ${\rm CO}_2$ acclimation treatment at the photosynthetic biochemical level.

This photosynthetic adaptation of rice to CO₂ acclimation treatment contrasts with some previous studies on other crops where no photosynthetic adaptive response to CO₂ acclimation treatment was observed when plants were introduced to a new. CO₂ environment (Acock et al., 1985; Ford and Thorne, 1967; Jones et al., 1985a; Mauney et al., 1979). However, these studies have dealt with ambient and superambient CO₂ treatments whereas the results in Table 2 indicate that the adaptive response to CO₂ acclimation treatment was greatest among the subambient treatments.

Expressed on a per unit leaf area basis, decreased photosynthetic rates of high CO₂ acclimated plants relative to ambient acclimated plants when measured at a common CO₂ level have been reported in other studies (Clough et al., 1981; Aoki and Yabuki, 1977; Kriedemann et al., 1976; Mauney et al., 1978). A similar acclimation response at

the canopy level for rice is found in the present study where long-term ${\rm CO}_2$ acclimation treatment resulted in small differences in total canopy leaf area.

In the ambient and superambient treatments, measurements of Pn during the afternoon were frequently lower than measurements made during the morning at similar levels of PPFD. An example of this decline can be seen in Table 2 by comparing the AM and PM values of Pn for the 330 and 660 treatments on day 66 and 67, respectively. Similar declines in photosynthetic rate have been associated with increases in assimilate concentrations (Mauney et al., 1979; Nafziger and Koller, 1976; Upmeyer and Koller, 1973) and increases in specific leaf weight (Chatterton, 1973) leading to the hypothesis that assimilate accumulation reduces leaf photosynthetic rate (Neales and Incoll, 1968). Although this idea of direct feedback control of photosynthesis by product inhibition is plausible, other evidence suggests a less direct mechanism involving hormonal control (Geiger, In contrast to the ambient and superambient treatments, the subambient treatments displayed essentially no decline in P_n from morning to afternoon when measured similar levels of PPFD. An example of this consistency Pn can be seen in Table 2 by comparing AM and PM estimates for the 160 μ mol mol⁻¹ treatment on days 62 and 65.

The linear photosynthetic light response and lack of tendency towards light saturation after the beginning of stem extension is attributed to the high plant population

used in this study and the erect leaf orientation of IR-30. This type of erect leaf orientation is a common morphological trait found in many of the modern, improved rice cultivars (Yoshida, 1981). Similar linear photosynthetic light response has also been reported for rice (Murata, 1961), cotton (Baker, 1965; Hesketh and Baker, 1967) and soybean (Sionit et al., 1984).

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SECTION V

Changes In Stomatal Density In Rice Grown Under Various CO₂
Regimes With Natural Solar Irradiance.

A.J. Rowland-Bamford, C. Nordenbrock, J.T. Baker, G. Bowes, and L.H. Allen, Jr.

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V. Changes In Stomatal Density In Rice Grown Under Various
CO₂ Regimes With Natural Solar Irradiance.

ABSTRACT

Rice (Oryza sativa L. cv. IR-30), grown under natural solar irradiance, was exposed to CO_2 concentrations ranging from 160 to 900 microliters CO_2 per liter air from 9 days after planting until maturity. Stomatal density was determined from leaf impressions at two growth stages: on leaf number 7 (31 days after planting), and on flag leaves (104 days after planting).

Increasing CO₂ concentrations resulted in a rise in stomatal density for leaves at both growth stages. The effect was greatest on the flag leaves, which exhibited a 55% increase in abaxial stomatal density (from 550 to 810 stomata per square millimeter) at 500 as compared with 160 microliters CO₂ per liter. For both leaf ages, the abaxial stomatal density was more influenced by increases in CO₂ than the adaxial surface. The flag leaf showed the greatest response between 160 and 500 microliters CO₂ per liter, respectively; above 500 there was no further significant change in stomatal density. The increase in stomatal density was largely the result of a rise in the number of stomata per row, although on the abaxial surface more rows across the leaf also contributed to the response. Flag leaf area was not significantly different among the CO₂ treatments, so

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the number of stomata per leaf followed similar trends to the stomatal density. This indicated the CO_2 effect was on stomatal, rather than leaf area, development. The response of stomatal density to rising CO_2 seems to be a species-dependent phenomenon, that varies with leaf surface and CO_2 range utilized.

INTRODUCTION

There has been a steady rise in atmospheric CO_2 since preindustrial times. The CO_2 has increased about 60 $\mu\mathrm{L}$ L⁻¹ air over the past 200 years (2). Furthermore, the concentration is expected to continue to rise. Some of the previous work examining the effect of these changes on vegetation has been short term or growth chamber studies (1, 7, 20). The plants were grown for only a short period under controlled CO_2 concentrations, under a lower photon irradiance or a different spectral distribution than that which occurs naturally. Both CO_2 and light cause adaptive responses in plants. Thus for predictive purposes, a more realistic approach is to control the CO_2 concentration over most or all of the life cycle, and use natural solar irradiance.

Major areas of research into responses of plants to CO₂ have included studies on photosynthesis and water relations (e.g. 5, 14, 18). In these areas, stomatal characteristics have a major influence on the response of a plant to the CO₂ treatment (11). Stomatal conductance, for example, is sensitive to a range of environmental stimuli, and several studies have concentrated on this aspect (11, 12). Similarly, variations in leaf morphology, including stomatal density, could have a considerable impact. Unfortunately, the literature dealing with stomatal characteristics appears contradictory. Two recent papers indicate that stomatal density generally decreases as the CO₂ is increased (19,



20), while other papers report no significant change (8, 16), or even increases for various species (13, 16). Because of this lack of consensus, and as part of a larger study of $\rm CO_2$ effects on the world's major crop plant, it was decided to examine stomatal density, stomatal number, and leaf area in rice grown for most of its life cycle under various $\rm CO_2$ concentrations and natural solar irradiance.

MATERIALS AND METHODS

Rice (Oryza sativa L. cv. IR-30) was grown in 1987 at Gainesville, Florida in the six outdoor chambers originally described by Jones et al (4, 5). These chambers have subsequently been modified for dewpoint control, and provided with improved covers. Dry bulb air temperature and dewpoint in each chamber was controlled at 31°C and 18°C, respectively. Rice was planted on 23 June at ambient CO2 partial pressure. Flooding was carried out when the seedlings reached the second leaf stage (9 days after planting). paddy water was maintained at 27°C. At this time, the CO2 was adjusted to one of six concentrations: 160, 250, 330, 500, 660, and 900 μ L CO₂ L⁻¹ air. Fertilizer was applied just prior to flooding at rates of 10, 9, and 9 g m⁻² for N, P, and K, respectively. Additional N, P, and K was applied at 23, 42, and 64 days after planting at rates of 4.8, 4.8, and 9.5 g m^{-2} , respectively.

Leaf impressions were made of the abaxial and adaxial surfaces at two growth stages: the first on 24 July on leaf number 7 (31 days after planting), and the second on 5 October (104 days after planting) on flag leaves. Imprints were made of leaves from each of the 6 chambers using clear nail varnish. On the first sampling date, 20 mm x 6 mm impressions were made at 100 mm from the ligule. At the second sampling date, the impressions (20 mm x 10 mm) were made 30

mm from the liquie. Each imprint was mounted immediately onto a slide and the number of stomata, number of rows of stomata, and the interveinal distances were determined in a random field under a light microscope at 400X magnification.

Statistical Analysis.

At the first sampling date, leaf impressions were made of 2 leaves from individual plants in each chamber on both surfaces. Each impression was counted in 5 random locations. At the second sampling date, leaf impressions were made of 6 leaves from individual plants, and each was counted in 5 random locations. A generalized regression model and Duncan's multiple range test were applied to the data.

RESULTS AND DISCUSSION

In rice leaves at both 31 and 104 days after planting, significant (F-value = 7.6, P < 0.005 for 31 days after planting and F-value = 19.4, P < 0.001 for 104 days after planting) increases in stomatal density were observed with increasing atmospheric $\rm CO_2$ concentrations during growth. For leaf number 7 (31 days after planting), this was especially evident between about 330 and 660 μL $\rm CO_2$ $\rm L^{-1}$, while a higher $\rm CO_2$ value had little or no further effect on stomatal density (Table I). For the abaxial leaf surface the trend was significant, but for the adaxial surface, although a similar trend was discernible, it was not statistically significant. The two surfaces of leaf number 7 exhibited similar stomatal densities at a given $\rm CO_2$ concentration (Table I).

For the later-developing flag leaves, the response was somewhat modified. Although the stomatal density increased with a rise in CO_2 , the CO_2 range for this effect was somewhat lower, being between 160 and about 500 $\mu\mathrm{L}$ CO_2 L^{-1} (Fig. 1). Values above 500 $\mu\mathrm{L}$ CO_2 L^{-1} did not result in further increases in stomatal density. The greatest effect of CO_2 on stomatal density occurred with the abaxial surface of the flag leaves, which showed a 55% increase between 160 and 500 $\mu\mathrm{L}$ CO_2 L^{-1} . At the higher CO_2 values this resulted in the abaxial surface having a greater stomatal density than the adaxial surface.

Table 1. Effect of atmospheric CO₂ on stomatal density on abaxial and adaxial surfaces of rice leaf #7. Means within columns with different letters are significantly different at P < 0.05 level by Duncan's multiple range test. Each value is the mean of two leaves, each measured at five areas. Standard error on the means are shown. Measurements carried out 22 days after imposition of CO₂ treatments.

*Significant at P < 0.05

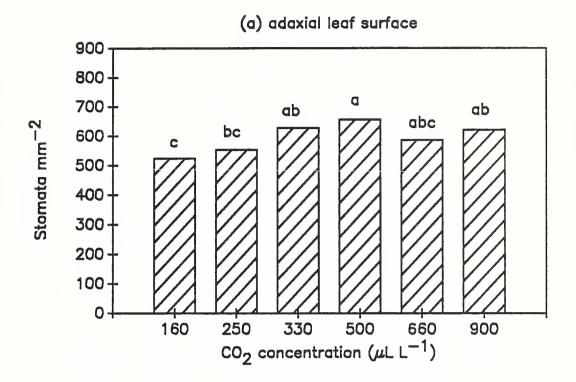
NS Not significant at P < 0.05

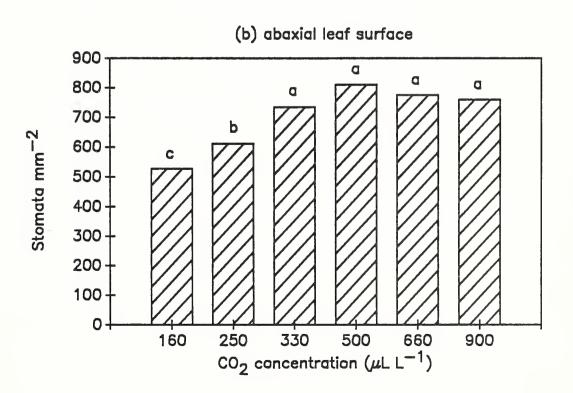
DF = 6

(L L-1	Stomatal (Stomat	Density a mm ⁻²)
	abaxial	adaxial
160	316 ± 15 ^b	328 <u>+</u> 68 ^a
250	317 ± 1 ^b	331 ± 47 ^a
330	302 ± 5 ^b	341 ± 33ª
500	339 ± 14 ^b	419 ± 47 ^a
660	459 ± 2ª	459 ± 2ª
900	488 ± 3 ^a	443 ± 11 ^a
F-value for CO ₂ effect	10.45*	ns

Figure 1.

Stomatal densities of flag leaves of rice grown under a range of CO_2 concentrations, on (a) adaxial surface and (b) abaxial surface. Different letters denote a significant difference at P<0.01 level; 6 leaves sampled and each surface of each leaf measured in five random locations. Measurements were made 14 weeks after the imposition of the CO_2 treatments.





The range of ${\rm CO}_2$ concentrations across which the plants were grown did not increase the leaf area, or cell expansion, of individual flag leaves (Table II). However, at the lower end of the ${\rm CO}_2$ range increases in ${\rm CO}_2$ did result in more stomata along the length of the leaf, and to a lesser extent, more rows of stomata across its width (Table II). For the abaxial side of flag leaves, this produced up to 60% more stomata at 660 as compared with 160 μL ${\rm CO}_2$ L^{-1} (Fig. 2). Thus for rice, changes in stomatal development, rather than in leaf area or cell expansion, seem to be responsible for the differences in leaf stomatal density and total stomatal number observed under increased ${\rm CO}_2$ concentrations.

These data show a differential effect of CO₂ on the two leaf surfaces. The cause of the different response of the leaf surfaces is unknown. It has been observed in cotton (Gossypium hirsutum L.) that a larger proportional increase in stomatal density occurs on the abaxial than on the adaxial surface (6). The stomatal density on the abaxial surface of soybean (Glycine max L.) and tomato (Lycopersicon esculentum Mill.) similarly has been shown to be more affected by environmental changes than that on the adaxial surface (9, 13).

The conflicting observations on stomatal density as a function of the CO₂ partial pressure used for growth, may be partly attributed to a species-dependent response. In

Table 2. Effect of various ${\rm CO}_2$ concentrations on leaf area and stomatal numbers of abaxial and adaxial surfaces of rice flag leaves.

which is the average count from five random field measurements. Values for leaf area level by Duncan's Multiple Range Test. Each value is the mean of six leaves, each of Means within columns with different letters are significantly different at P < 0.05 are means of 8-10 measurements and the standard error on the means are shown. Measurements carried out 95 days after imposition of ${\rm CO}_2$ treatments.

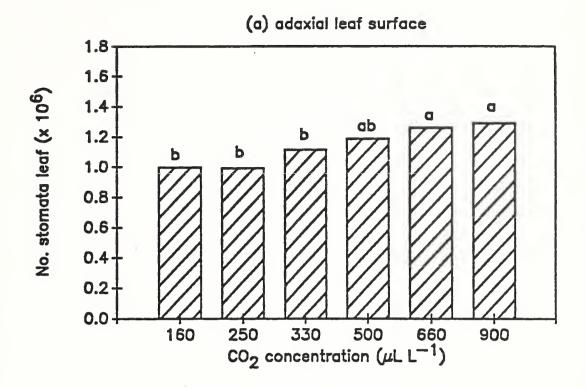
30 11 NS = Not significant at P < 0.05, DF * Significant at P < 0.05 level.

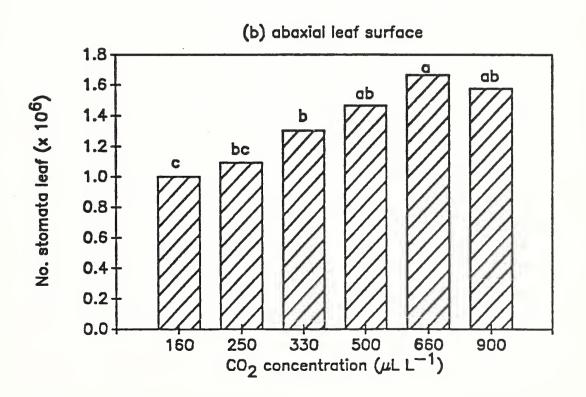
$\begin{pmatrix} co_2 \\ L L^{-1} \end{pmatrix}$	leaf area (cm ²)	number stomata along 1 mm row length	ata along length	number of rows across 1 mm of leaf width	ws across f width
		abaxial	adaxial	abaxial	adaxial
150	19.0 ± 1.3ª	23.1 ± 0.9 ^c	24.0 ± 0.7°	27.9 ± 1.0 ^b	28.1 ± 1.7ª
250	17.9 ± 2.2ª	24.6 ± 0.9bc	25.8 ± 0.8bc	32.1 ± 2.1ab	25.6 ± 2.4ª
330	17.8 ± 1.1ª	26.2 ± 1.5abc	27.2 ± 1.1abc	31.5 ± 0.8ab	28.8 ± 1.3ª
200	18.1 ± 1.2ª	28.2 ± 1.1ª	28.0 ± 1.1ab	35.3 ± 1.8ª	29.3 + 2.4a
099	21.5 ± 2.5ª	26.0 ± 0.6abc	25.8 ± 0.8bc	34.8 ± 1.3a	28.2 ± 1.7ª
006	20.7 ± 1.9a	26.8 ± 0.9ab	29.4 ± 1.1a	32.3 ± 1.3ab	25.9 ± 0.7ª
F-value for CO ₂ effect	S	2.96*	3.60*	3.26*	NG

FIGURE 2.

Effect of atmospheric CO₂ concentration on the number of stomata on rice flag leaves: (a) Adaxial surface and (b) abaxial surface. Different letters denote significant differences at P<0.01 levels, rest of legend as for Fig. 1.

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an examination of herbarium specimens over the past 200 years, Woodward (19) found a 40% decrease in stomatal density for eight arboreal species, which was supported by growth chamber studies of several plants under varying CO2 partial pressures (19, 20). A similar decrease was reported earlier for tomato (10). In contrast, a comparative study of historical and recent stomatal density data for over 200 species, produced no statistically significant evidence of an overall change in the stomatal density of plants over the past 120 years (8). However, some individual species did show substantial positive or negative changes over the observation period. For cotton, bean and soybean, growth experiments under various CO2 partial pressures indicate the stomatal density increases under elevated CO2 (6, 13, 16). Data for these three species are consistent with the response of rice in the present work.

As observed in this study, the ${\rm CO_2}$ concentration range influences the stomatal density response, with the effects diminishing at high (>500-600 μ L ${\rm CO_2}$ L⁻¹) concentrations (10, 16, 20). The response also varies with the developmental stage, and leaf surface under investigation.

Rice is unusual in terms of stomatal density and leaf morphology. With over 500 to 600 stomata $\rm mm^{-2}$ on each leaf surface, it had the highest stomatal density of 19 warm-climate species examined (2), and of all major crops (17). By way of comparison, maize (Zea mays L.) and soybean only exhibit between 62-104 and 79-373 stomata $\rm mm^{-2}$, respective-

ly. In the present study, leaf number 7 and the later-developing flag leaf averaged 300-350 and 650-750 stomata mm⁻², respectively at 330 μ L CO₂ L⁻¹, while at 500 μ L CO₂ L⁻¹ the flag leaf stomatal density rose as high as 810. Accompanying the very high stomatal density, rice also has a large leaf area, low leaf thickness, and a high surface area to volume ratio for the mesophyll cells (17). The leaf morphological characteristics produce a high conductance to CO₂ (15), and water vapor (2). These features appear to be advantageous under the hot, humid, and cloudy environment of the original rice growing areas, by potentially maximizing both photosynthesis and transpirational leaf cooling under these conditions (17).

Variation in stomatal density occurs within varieties of Oryza sativa. The indica varieties, like IR-30, usually have higher stomatal densities than those of japonica. The indica varieties are thermally adapted to the original rice growing areas, whereas various leaf characteristics of japonica varieties have enabled rice cultivation to expand into cooler, drier habitats (17). It has been suggested that the differences in leaf morphological characteristics between japonica and indica varieties reflect the environments in which the two species evolved (17). If this is the case, then the increase in stomatal density of O. sativa cv IR-30 under elevated CO2 might alter its climatic response. To predict potential acclimation effects, these changes in

stomatal density need to be evaluated in relation to further measurements involving photosynthetic water use efficiency, transpirational leaf cooling, and leaf specific density.

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SECTION VI

Rubisco Activity And Rubisco Protein Content in Rice Grown Under Various ${\rm CO_2}$ Concentrations

A.J. Rowland-Bamford, L.H. Allen, Jr., and G. Bowes.

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VI. Rubisco Activity And Rubisco Protein Content in Rice Grown Under Various CO₂ Concentrations

ABSTRACT

The effects of long-term, season long exposure to a range of atmospheric CO₂ concentrations on the activity, amount, dark inhibition and activation state of ribulose-1,5,-bisphosphate carboxylase (rubisco) was studied in rice (Oryza sativa L. IR-30). Rice was grown in outdoor computer-controlled environment chambers under natural solar radiation with CO₂ concentration maintained at 160, 250, 330, 500, 660 and 900 µmol⁻¹ air until maturity.

The activity of rubisco declined with increasing CO₂ concentration in the atmosphere. The response was more marked when rates were expressed on a leaf total soluble protein basis than on a rubisco protein basis. This decline in rubisco activity was accompanied by a decrease in the amount of rubisco protein as a percent of the total soluble protein in the leaf. Samples taken from pre-dawn to dusk demonstrated that rice rubisco activity showed dark inhibition of the CO₂/Mg²⁺-activated enzyme, indicating that rice belongs to the group of species that accumulate the inhibitor 2-carboxyarabinitol 1-phosphate in the dark. Activation state of the rubisco enzyme was unchanged by the atmospheric CO₂ regime except at the highest concentration used where it declined from the usual 100% to 76%.

These results indicate that acclimation of rice to the

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atmospheric ${\rm CO}_2$ conditions involves the modulation of the amount of rubisco protein and activity in the leaf.

INTRODUCTION

The global atmospheric CO2 concentration, presently at 350 µmol mol⁻¹, is increasing and could double by the end of the next century. The CO2 concentration in the atmosphere at present is generally limiting to the photosynthesis of C3 plants (Pearcy & Bjorkman, 1983). Increasing the CO2 levels to which plants are exposed decreases the rate of photorespiration as well as provides more substrate for photosynthe-Sharkey (1989) calculated that a doubling of the sis. present atmospheric CO2 concentration would result in a 50% decrease in the ratio of photorespiration to photosynthesis. The enzyme responsible for initiating C3 photosynthesis, ribulose 1,5-bisphosphate carboxylase / oxygenase (E.C. 4.1.1.39; Rubisco), plays a pivotal role in both photosynthetic and photorespiration pathways. It has been the focus of much attention regarding the regulation of the rate of carbon entering the photosynthetic pathway. Rubisco catalyzes the carboxylation of RuBP (ribulose 1,5 bisphosphate), the first step in the photosynthetic carbon reduction pathway.

In addition to its importance as a carboxylase, rubisco protein represents a considerable storage pool of protein
and nitrogen, which can be remobilized, especially during
senescence. This enzyme can represent up to 65% of the total
soluble protein in leaves (Ellis, 1979).

Long-term exposure to an atmosphere enriched with CO2

has been reported to result in acclimation in the rates of growth (Peet, 1986; Knight and Mitchell, 1988), photosynthesis (Peet et al, 1986), and rubisco activity (Peet et al, 1986; Spencer and Bowes, 1986) in a variety of species although the acclimation of rubisco activity was not observed in soybean (Campbell et al, 1988). In many studies it is unclear whether the changes in rubisco activity result from reduced quantity of rubisco protein or a decrease in the activity of the existing enzyme or both.

This work attempts to examine the mechanism underlying the response of rice to long-term exposure to CO_2 . Despite our understanding of the short-term responses of photosynthesis to elevated CO_2 , much less known about the mechanisms by which photosynthesis responds to long-term CO_2 elevation. In order to elucidate some of these questions, the activity, amount and activation state of rubisco were analysed along with leaf chlorophyll and total soluble protein content.

MATERIAL AND METHODS

GROWTH CONDITIONS.

Rice plants (<u>Oryza sativa</u> L. IR-30) were grown from seed sown on 23rd June, 1987, in 6 outdoor, sunlit, computer-managed, controlled-environment plant growth chambers. Further chamber details may be found in Jones <u>et al</u> (1984, 1985) and Section IV. The average dry bulb air temperature was 31°C with a dewpoint temperature of 18°C. The plants were flooded when the seedlings reached the 2nd/3rd leaf stage, and the paddy temperature was maintained at 27°C. Two days later the CO₂ level in each chamber was adjusted to one of 6 concentrations; 160, 250, 330, 500, 660 & 900 µmol mol⁻¹ air until maturity (111 days after planting).

(A) RUBISCO ACTIVITY

Rubisco activity as reported here refers to the carboxylase activity of the enzyme. Flag leaves were sampled at mid-day, 86 days after planting, from plants exposed to range of CO_2 concentrations (160 - 900 μ mol CO_2 mol^{-1}) when solar photosynthetic photon flux density (PFD) was greater than 1000 μ mol photons m^{-2} s⁻¹, and at intervals throughout the day from predawn to dusk from plants exposed to 160, 330, and 660 μ mol CO_2 mol^{-1} , 99 days after planting. Samples were frozen immediately in liquid N_2 (LN₂).

Rubisco activity was assayed in two ways to give (i) initial (in vivo) and (ii) total activity. The former involves the extraction of liquid N_2 -frozen leaf tissue (1 g) on ice in ice-cold medium, similar to that described by Makino et al (1987), containing 100 mM HEPES (pH 7.8), 5 mM DTT, 25 mM MgCl₂, 1 mM EDTA, 12.5% (v/v) glycerol, and 10 mM sodium isoascorbate. The extract was immediately microfuged for 1 min and then assayed at 25°C in a medium containing 100 mM bicine, 25 mM MgCl₂, 10 mM isoascorbate, 5 mM DTT, 10 mM NaH¹⁴CO₂ (2.05 GBq mmol⁻¹) and 1 mM RuBP. The reaction was started with an aliquot of the centrifuged crude leaf extract and stopped with glacial acetic acid. The amount of 14 CO₂ fixed was determined as acid stable products by liquid scintillation spectrometry.

Total rubisco activity was determined after a 5 min incubation period of an aliquot of the extract in the assay medium described above with HCO_3^- and Mg^{2+} but without the addition of RuBP. This treatment serves to fully activate the available enzyme sites. A time course confirmed that a 5 minute incubation was sufficient to fully activate rubisco enzyme from leaf samples taken in the light (data not shown). The reaction was initiated with the injection of 50 ul 10 mM RuBP, into the total reaction volume of 500 ul, giving a concentration of 1 mM RuBP. The reaction was terminated by the addition of glacial acetic acid. Activation state or percent activation [100 (initial/total activity)] is used to describe the amount of enzyme existing in the

leaf in the active state.

(B) RUBISCO PROTEIN

The amount of rubisco protein in the crude leaf extract was measured using SDS-gel Page electrophoresis (Laemmli, 1970) as described by Servaites et al (1984). Partially purified spinach rubisco protein (Sigma) was used as a standard for calibration. Rubisco protein was quantified by excising and eluting in 1% (v/v) SDS the Coomassie brilliant blue R stained bands of the large and small subunits. Following centrifugation at 7,000g for 3 min, absorbance was read at 600 nm.

(C) TOTAL SOLUBLE PROTEIN AND CHLOROPHYLL CONTENT.

Total soluble protein of aliquots of the leaf extract was determined with the dye-binding method (Biorad, Richmond, CA) using bovine serum albumin as the standard. Chlorophyll was extracted in 100% (v/v) ethanol overnight in the dark at 4°C, then spun in a bench centrifuge for 5 min. Total chlorophyll content was determined using the method of Winterman and DeMots (1965), measuring absorbance at 654 nm.

RESULTS

Initial and total activities of rubisco in rice declined with increasing CO₂ concentration supplied during the growing season (Fig. 1). The response was greater when the rates were expressed on a leaf total soluble protein basis than on a rubisco protein basis. Total rubisco activity expressed in terms of total soluble protein dropped 47.5% with an increase of CO₂ from 160 to 900 µmol mol⁻¹. On a chlorophyll basis the decrease in rubisco activity with increasing CO₂ was large (Fig. 2). This decline was due mainly to a decline in the chlorophyll content of the leaves (Fig. 3).

The concentration of rubisco protein in rice leaves also decreased with increasing CO_2 (Fig. 4). The amount of rubisco protein expressed on a leaf total soluble protein basis decreased by 31% with an increase in CO_2 concentration from 160 to 900 µmol mol⁻¹.

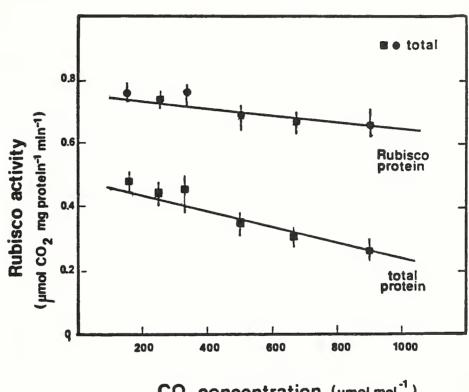
The percent activation of rubisco enzyme in the light remained near 100% at all CO_2 concentrate except 900 µmol mol^{-1} , where activation dropped to 76% (Table 1).

The changes in rubisco activity through the period from pre-dawn to dusk are shown in Figures 5 and 6. As demonstrated before, rubisco activity was lower in rice leaves grown at the higher ${\rm CO}_2$ concentrations. At PFD above 500 μ mol photons m⁻² s⁻¹, rubisco activity was higher in plants grown at subambient ${\rm CO}_2$ concentrations than at

Figure 1

The response of rubisco activity to atmospheric ${\rm CO_2}$ concentration. Rates are expressed on a leaf total soluble protein basis and on a rubisco protein basis. Values are means \pm S.E.

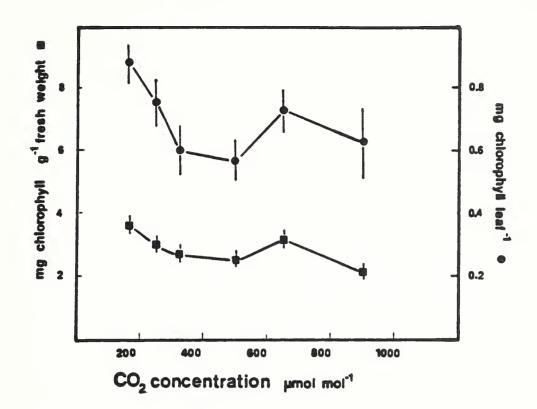
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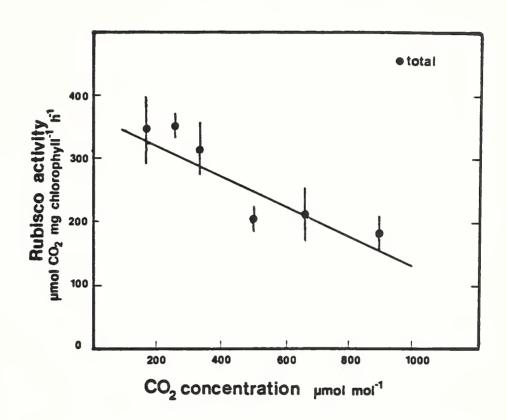
CO₂ concentration (µmol mol⁻¹)



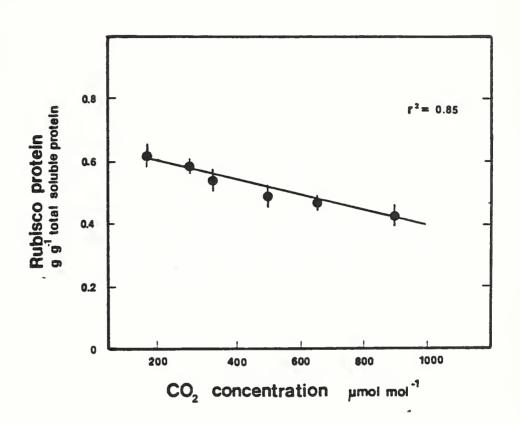
The effect of atmospheric ${\rm CO_2}$ on rubisco activity expressed on a chlorophyll basis. Values are means \pm S.E.



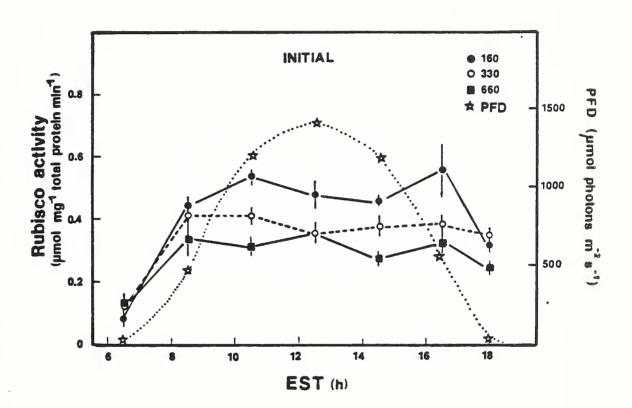
The effect of atmospheric CO_2 levels on chlorophyll levels in rice 'leaves. Values are means \pm S.E.



The effect of atmospheric CO_2 on the amount of rubisco protein expressed on a leaf total protein basis. Values are means \pm S.E.



Initial rubisco activity, 99 days after planting, from predawn to dusk of rice flag leaves grown at 160, 330 660 umol CO_2 mol⁻¹ air during the growing season. Values are means \pm S.E. Solar photosynthetic photon flux density (PFD) is also shown for the sampling day.



Total CO_2/Mg^{2+} -activated rubisco activity, 99 days after planting, from predawn to dusk of rice flag leaves grown at 160, 330 660 umol CO_2 mol⁻¹ air during the growing season. Values are means \pm S.E. Solar photosynthetic photon flux density (PFD) is also shown for the sampling day.

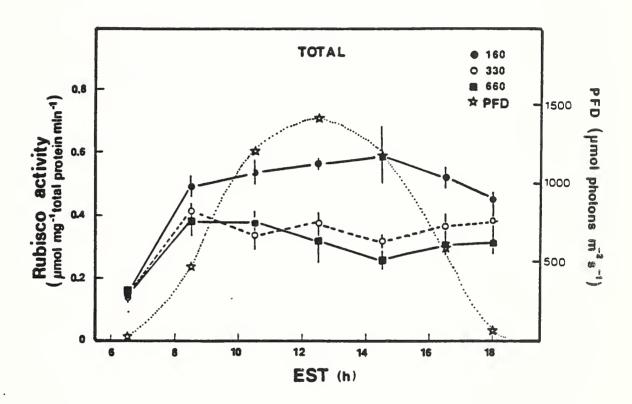


Table 1. Activation states of rubisco in rice flag leaves grown under various ${\rm CO}_2$ concentration in light (PFD > 500).

CO ₂ concentration (µmol mol ⁻¹)	% Activation ⁺
160 250	101.2 ± 3.9* 100.0 ± 4
330	100.0 ± 4.2*
500 660	97.6 ± 3 103.1 ± 6.6*
900	76.0 ± 4

^{*} n = 4; rest n = 2

^{+ 100 (}initial activity/total activity)

ambient and superambient concentrations.

The total CO_2/Mg^{2+} -activated rubisco activity in the dark was low compared to that in the light (Fig. 6). Dark inhibition (100 * total activity in predawn/mean total activity in light; PFD > 500) was 28%, 33%, and 36% in the subambient, ambient, and superambient treatments, respectively. Furthermore, the drop in total rubisco activity at dusk was slow. Total activity was still high 1.5 hours after PFD had begun to fall. This contrasts to initial activity of rubisco, where rates fell with decreasing PFD at dusk, especially in the subambient CO_2 treatment (Fig. 5).

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DISCUSSION

The activity of rubisco enzyme in rice declined with increasing CO2 concentration supplied during the growing season (Figs. 1 and 2). The response was much greater when the rates were expressed on a leaf total soluble protein basis than on a rubisco protein basis. The drop in activity was accompanied by a decrease in the concentration of rubisco protein expressed on a total protein basis (Fig. 4). Thus, a major cause of the decline in rubisco activity is a drop in the amount of rubisco protein, relative to other proteins in the leaf. Peet et al (1986) also found that rubisco activity in cucumber (Cucumber sativus L.) decreased at higher CO₂ concentrations (1000 µmol mol⁻¹) as compared to 350 µmol mol⁻¹. Water hyacinth (Eichhornia crassipes [Mart.] Solms) showed a similar response (Spencer and Bowes, 1986). However, the response of rubisco protein content was not examined in these earlier studies.

The activity of rubisco per crude protein in rice leaves ranged from 0.202 to 0.505 µmol mg⁻¹ total protein min⁻¹, which is within the range of previous studies (Makino et al, 1988). Also, the specific activity was similar to published ranges (Makino et al, 1983; Shieh & Liao, 1988), with rates of 0.6 to 0.8 µmol mg⁻¹ rubisco protein min⁻¹. Furthermore, the range for the ratio of rubisco protein to total soluble protein agreed with those of Makino et al (1987).

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Rubisco must be activated by the reversible carbamylation of a specific lysine residue on the enzyme with CO2 and Mg²⁺ (see review by Miziorko and Lorimer, 1983). This is catalysed in vivo by the enzyme rubisco activase (Portis et al, 1986; Salvucci et al, 1986) in the light. The activation state is a measure of the proportion of the enzyme in an active form, i.e. the percentage of sites which are apparently carbamylated in vivo, and not bound by an inhibitor. In rice, this remained unchanged over most of the range of CO2 treatments used in this study. However, at 900 µmol mol⁻¹ a drop in activation state was observed. This is similar to that found by Sage et al (1989) who compared five C3 species grown at 300 or 900-1000 $\mu mol\ CO_2\ mol^{-1}$. All five showed significant decreases in percent activation. Short-term (min) increases in CO2 concentration (360 to 1600 µmol mol 1) also resulted in decreases in rubisco activation state (Sage et al, 1988). However, in this study on rice, using a range of CO2, percent activation only dropped in the 900 µmol mol⁻¹ treatment. It is possible that with the doubling of present CO2 concentration predicted over the next century that rice will not show a change in activation state. can be calculated, from rubisco content and activation state, that in plants grown at 160 µmol CO2 mol-1 61% of the total leaf protein was catalytically effective rubisco enzyme, at 330 µmol mol⁻¹ only 48% was catalytically effective and at 900 μ mol mol⁻¹ only 32% of total leaf protein was

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catalytically effective enzyme.

Sage et al (1989) proposed that in CO_2 -enriched atmospheres, activation states should recover due to the degradation of rubisco. If the leaf does not fully acclimate, the rubisco will remain partially deactivated. This could be the case for rice at the 900 μ mol mol⁻¹, whereas 500 and 660 have acclimated succesfully.

These results indicate that some level of 'acclimation' to the prevailing CO₂ concentration in the atmosphere, may be taking place in rice leaves over the period of the growing season. This parallels the response in species such as water hyacinth (Spencer & Bowes, 1986), but contrasts to that shown by soybean (Campbell et al, 1988) where rubsico activity remains unchanged on a total soluble protein basis although it decreases somewhat on a chlorophyll basis (Vu et al, 1983).

The changes in rubisco activity through the period from pre-dawn to dusk further showed differences between rice grown at sub-ambient, ambient and superambient ${\rm CO_2}$ concentrations. At PFD above 500 µmol photons ${\rm m^{-2}~s^{-1}}$ during the middle of the day, enzyme activity was higher in plants grown at subambient ${\rm CO_2}$ levels than at ambient and superambient concentrations. The low ${\rm CO_2/Mg^{2+}}$ -activated rubisco activity in the dark (<40% of that in the light) shows that rice belongs to the group of species that is inhibited in the dark even though the enzyme is fully activated. This dark/light modulation of rubisco has been shown to be

present in a variety of plants (Vu et al, 1984; Seemann et al, 1985; Servaites et al, 1986). The inhibition was highest for the leaves grown at the lower CO2 concentrations. Of 37 species examined by Servaites et al (1986), 23 showed dark inhibitions of <75% and 3 showed dark inhibitions of <50% of that in the light. Phaseolus vulgaris had the largest dark inhibition at 10% (i.e. dark activity 10% of that in light). This inhibition of rubisco activity in the dark as compared to the light of ${\rm CO_2/Mg^{2+}}$ - incubated extracts has been attributed to the presence of a phosphorylated inhibitor, 2carboxyarabinitol-1-P (CA-1-P), which binds tightly to the enzymes active sites (Gutteridge et al, 1986; Berry et al, 1987). This compound accumulates at low irradiance and is mostly below detectable levels at high irradiance (Servaites, 1985: Seemann et al, 1985). The percent dark inhibition of that in the light of CO,/Mg²⁺-activated (total) enzyme activity is a measure of the degree of inhibition by CAPP and this is greatest in rice leaves at the higher CO2 concentrations.

Taking leaf samples from predawn to dusk demonstrated that in rice the drop in activity at dusk was slow whiletotal activity was still high 1.5 hours after PFD started to fall (Fig. 6). However, initial activity in all 3 CO₂ treatments began to decrease at dusk, especially in the subambient treatment (Fig. 5). This contrasts with the response at dawn where both initial and total activity increased as ir-

radiance increased. Servaites et al (1984) also found that while initial and total activity of soybean rubisco both increased with increasing irradiance at the beginning of the day, total activity declined in the dark at a much slower rate than inital activity. This indicates that the effect of light on the two activities may be mechanistically different. Furthermore, Salvucci and Anderson (1987), using protoplasts, found similar changes in activation state and total activatable rubisco activity; both increased after increased illumination. However, upon darkening, there was immediate decrease in activated state of rubisco, (i.e. initial activity) and deactivation was slower than activation. A slight decrease in total activity occurred upon darkening but the rate was considerable slower than the drop in activation state.

Since rubisco constitutes such a major sink for N in the leaf, the drop in content could have a great effect on N partitioning within the leaf. This enables reallocation of resources to either other components of photosynthetic processes or to non-photosynthetic processes. Therefore, the drop in rubisco content measured with increasing CO_2 (Fig.4) would result in reallocation of rubisco protein N which is not required due to the increase in substrate CO_2 . Therefore in rice, the percentage of N invested in rubisco protein decreased as the CO_2 concentration in the atmosphere increased. Sage et al (1989) obtained similar results with 2 of the 5 species tested. Increasing the N supply may also in-

crease the ratio of rubisco protein to total soluble protein in rice leaves although the ratio is independent of N nutrition at full leaf expansion (Makino et al, 1984). This indicates that the ratio of rubsico protein to total soluble protein can be regulated by the plant to maximise the use of resources.

Rice responded to an increase in the atmospheric CO2 concentration from 160 µmol CO₂ mol⁻¹ with increasing rates of photosynthesis but these rates remained relatively constant above 500 µmol CO2 mol-1 air (see Section IV). This contrasts with previous studies in this lab on soybean where the rates were still increasing at 900 µmol mol⁻¹ (Campbell et al, 1988). The leveling off of the response of photosynthetic rates in rice to increasing CO₂ above 500 µmol mol⁻¹ may be due a balance between the increased availability of substrate (CO2) and a decline in the amount of active enzyme in the leaf, with a possible readjustment in activation state. No such acclimation responses were observed in soybean (Campbell et al, 1988). Further evidence for acclimation is provided by determining photosynthetic rates of plants in CO2 environment of different concentration to that used during growth. Imai and Matura (1978) found that rice grown at 160 μ mol mol⁻¹ CO₂ when switched to 350 μ mol mol⁻¹ exhibited higher photosynthetic activity than rice grown at 350 µmol mol⁻¹ throughout the experiment. The converse was true for those grown at 1000 μ mol mol⁻¹ and transferred to

ambient CO_2 concentrations. Peet et al (1986) obtained a similar response with cucumbers, comparing concentrations of 350 and 1000 μ mol mol^{-1} . Neither study measured rubisco protein content although Peet et al (1986) did observe a decrease in rubisco activity in the high CO_2 treatment after vegetative growth. The degradation of rubisco protein could offer an explanation, in addition to the drop in rubsico activity, for the acclimation response. Rice acclimation to higher CO_2 concentrations, therefore, involves a decrease in the resources allocated to protein, especially rubisco, and an increase in the allocation to carbohydrates (see Section VII).

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SECTION VII

Effect of CO₂ Concentration During The Growing Season on Carbohydrate Status and Partitioning in Rice

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VII. Effect of CO₂ Concentration During The Growing Season on Carbohydrate Status and Partitioning in Rice

ABSTRACT

The atmospheric carbon dioxide (CO2) concentration has been rising and is predicited to double sometime during the next century. The objectives of this investigations were to determine the long-term effects of different atmospheric CO2 concentration on certain aspects of carbohydrate status and partitioning in rice (Oryza sativa L. IR-30), a globally important crop plants. Rice plants were grown season-long in outdoor, naturally sun-lit, computer controlled environment, plant growth chambers in CO2 concentrations of 160, 250, 330 (ambient), 500, 660, and 900 µmol CO₂ mol⁻¹ air. Increasing CO2 concentration resulted in an increase in total nonstructural carbohydrate (TNC) concentration in leaf blades, leaf sheaths and culms, but did not increase the carbohydrate concentration in the grain at maturity. The increase in vegetative carbohydrate concentration was observed from the 160 to 500 μ mol mol⁻¹ treatments and leveled off at CO_2 treatments above this concentration. Leaf sucrose and starch concentrations increased with increasing CO2 concentration early in the season and showed similar leveling off at 500 µmol mol⁻¹. Furthermore, early in the season, the ratio of sucrose to starch also responded to CO2 concentrations, dropping exponentially with increasing ${\rm CO}_2$ concentrations. In leaf blades, the priority between the partitioning of

carbon into storage carbohyhdrates or into export changed with developmental stage and CO2 concentration. Early in season, at ambient and superambient CO2 concentrations, the priority was for export in contrast to subambient conditions where the priority was for storage. Later, the priority was for storage, at all CO2 concentrations except 160 µmol mol 1 . Early in the seaon, the rate of export was positively correlated to the photosynthetic rate. The rate of carbohydrate input into the panicle was affected by developmental stage and CO2 treatment. Furthermore, the contribution to the grain of carbohydrates stored before heading increased with increasing CO₂ concentration up 500 umol mol⁻¹ and leveled off at CO2 concentrations above this. At final harvest, the plants maintained a consistent partitioning of nonstructural carbohydrates into all plant parts, although the total amount of carbohydrates available increased with increasing CO₂ up to 500 umol mol⁻¹. Although vegetative parts showed increases in carbohydrate concentration, this was not reflected in grain carbohydrate concentration at final harvest.

INTRODUCTION

The coordination of transformation and transportation of carbohydrates within plants determines partitioning - the pattern in which material appears in different chemical forms and in different tissue and organs (Lang and Thorpe, 1983). A fuller understanding of this activity could lead to greater appreciation of how plants will cope with the increase in the supply of carbon in plants due to the increase of CO₂ in the atmosphere, expected over the next century (Baes et al, 1974; Trabalka et al, 1986).

The enhancement of growth and yields by CO₂ enrichment has been reported for many species (Kimball, 1983; Cure, 1985). Indeed, for a doubling of the present day CO₂ concentration, under optimum conditions, plant productivity and yields rise on average by about 30% for most crops (Kimball, 1983). The regulation of C fixation and partitioning of the photoassimilate to each plant part and between the different chemical forms is of primary importance to plant productivity. The primary end products of photosynthesis are starch and sucrose. Starch is deposited during the during the day exclusivley in the chloroplasts (Preiss, 1982). Sucrose is synthesized in the cytosol, can be stored in the vacuole and constitutes the main form of reduced carbon translocated from leaves to growing points in the plant such as the panicles in rice.

Increasing atmospheric CO2 results in an increase in photosynthesis in rice (see Section IV). This would result in increased availability of C for carbohydrate synthesis. However, a leveling off of the response of rice photosynthesis to rising CO_2 was observed above 500 μ mol mol⁻¹. Other species also lose their response to increasing CO2 when subjected for an extended period to high concentrations of CO2 (Hinkleton and Jollife, 1985; Peet et al, 1986). One of the hypothesis most often proposed to account for this is the buildup of starch and sugars, which results in feedback inhibition of photosynthesis (Thomas et al, 1975; Mauney et al, 1979; Azcon-Bieto, 1983; Nafziger and Koller, 1976; Peet and Kramer, 1980). The accumulation of sucrose and starch could be important in the control, directly or indirectly of photosynthesis in plants grown under CO2 enrichment. objectives of this study were to determine the effects of CO2 concentration in the atmosphere on the concentration and amount of total non-structural carbohydrates, starch and sugars throughout the growing season and to examine accompanying daily patterns in these pools. Partitioning of carbohydrate to plant parts under various CO2 concentrations during the growing period was also investigated.

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MATERIALS AND METHODS

GROWTH CONDITIONS

A detailed description of the controlled environment chambers in which the rice was grown under various CO2 regimes $(160 - 900 \, \mu\text{mol mol}^{-1})$ from sowing $(23 \, \text{June } 1987) \, \text{un}$ til maturity is provided by Jones et al (1984) and in Sections II to IV. Plants were destructively sampled at 30, 44, 58, 86 and 110 days after planting, at or after midday. In addition, at 19 and 71 days after planting (DAP) samples were taken in the morning (before 09:30) and in the afternoon (after 15:30). Dry weights were determined for each plant part after oven drying. At 19, 44 and 71 DAP, 20 plants were harvested from each chamber, while at 86 DAP, 10 plants were sampled. Half the plants were collected from one side of the chamber and the other half from the other side. At final harvest 45 plants were sampled from each chamber, 15 from the east side, 15 from the middle and 15 from the west side. At 30 and 58 DAP 9 plants were harvested from each chamber and pooled.

CARBOHYDRATE ANALYSIS

Total non-structural carbohydrates.

Total non-structural carbohydrate (TNC) concentration was determined as described by Allen et al (1988). Distilled water (5ml) was added to dried ground plant material (0.1g) and boiled for 10 mins. After cooling 5ml of 0.2M acetate buffer (pH 4.5) was added with 1ml of enzyme mix. This con-



sisted of 45ml distilled water, 5ml of 0.1M acetate buffer (pH 4.5), 2.5ml invertase (yeast concentrate in glycerol; 350 units/ml), 1.25g amyloglucosidase (from Rhizopus; Sigma) and 0.1g thymol. The reaction mix was then incubated at 48°C for 48 hrs and shaken continuously. Following filtration, the filtrate was assayed for reducing sugars (as glucose equivalents) after appropriate dilution using the Nelson-Somogyi modified method (Spiro, 1966).

For TNC analysis, 2-3 extractions were made from each pooled sample. The TNC concentration was determined twice for each extraction. Only samples taken after 12:00hrs were included in the statistical analysis. The data were subjected to an analysis of variance by the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS). The significant differences between the means was determined using Duncan's Multiple Range test.

Soluble Sugar analysis.

Dried plant material (0.1g) was extracted at least 3 times with 80% (v/v) ethanol at 95°C for about 1 hour for each extraction. It was confirmed that this was sufficient to fully extract nearly all the ethanol-soluble sugars in the plant tissue (data not shown). This extraction removes the hexoses, sucrose and low degree of polymerization fructans (<5) from the plant tissue. A total volume of 30 ml ethanol was used. An aliquot of the ethanol was then ana-

lyzed, after suitable dilution, for sucrose and fructose using rescorcinol (Ashwell, 1957). Sucrose was measured in an aliquot by the addition of 1ml rescorcinol (0.1% w/v in glacial acetic acid) and 7ml of conc. HCl after boiling for 10 mins with 1M NaOH to destroy free fructose. The tubes were incubated at 80°C for 30 mins and absorbance read at 520 nm. Fructose was calculated from the difference between the fructose equivalent concentration in samples heated with NaOH and those heated with distilled water (i.e. fructose equivalents from sucrose and fructose - fructose equivalents from sucrose only).

Starch analysis

The pellet remaining after the ethanol extraction described above was dried overnight in an oven at 60°C. After the addition of 10ml distilled water, the pellets were boiled for 10 mins. The tubes were then incubated at 41°C for 48 hrs with 0.2M acetate buffer (10ml; pH 4.5) and 10 ml of 0.5% (w/v) amyloglucosidase (from Rhizopus, Sigma). This procedure extracts starch and fructans with a degree of polymerization of >5. The glucose concentration in the supernatant was determined using the Nelson-Somogyi test (Spiro, 1966) and converted to starch equivalent by comparing to standard starch samples run in conjunction with the plant material. Fructan content was measured in the supernatant using rescorcinol described as above.

Estimation of export rates of carbohydrates from leaf blades.

Maximum rates of net canopy photosynthesis were estimated from regression equations fitted to each days data. The PFD averaged 1263 and 1007 µmol photons m^{-1} s⁻¹ for 19 and 71 DAP respectively. Net photosynthetic rates were converted from µmol CO_2 fixed m^{-1} ground area s⁻¹ to mg $\mathrm{CH}_2\mathrm{O}$ g⁻¹ leaf dry weight day⁻¹ by multiplying by 0.68 (the molar ratio of the two forms of carbon) and by 44 to convert moles to grams. Ground area was converted to leaf dry weight using the known g DW plant⁻¹ and the fact that 235 plants were sown per m^2 of ground area. Estimates of mass carbon export rates were then based on the difference between net $\mathrm{CH}_2\mathrm{O}$ production rates by photosynthesis and the change in total leaf TNC concentration (mg g⁻¹ DW d⁻¹) at 19 DAP (Borland and Farrar, 1987). This was calculated by the difference in TNC between pm and am samples.

RESULTS

TOTAL NONSTRUCTURAL CARBOHYDRATE CONTENT

The amount of nonstructural carbohydrate (mg plant⁻¹) in the above ground portion of rice (leaf blades, leaf sheaths, culms, grain and chaff) increased with increasing CO₂ treatment (Figs. 1A-D). At 30 DAP, the plant TNC content increased almost 6 fold as CO₂ increased from 160 to 900 umol mol⁻¹. As the season progressed, the TNC content in the plants increased. Furthermore, the response of TNC content to increasing CO₂ began to level off at CO₂ concentrations above 500 umol mol⁻¹ CO₂. At final harvest, the plant carbohydrate content increased with increasing CO₂ from 160 up to 500 umol mol⁻¹ but raising the concentration of CO₂ above this level had little effect on the carbohydrate content of the rice plants (Fig. 1D).

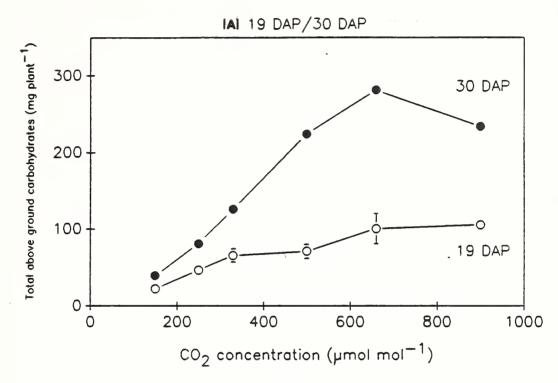
From 19 DAP to final harvest, the amount of above ground carbohydrate in 160 umol mol^{-1} CO_2 treatment as % of that in ambient CO_2 conditions ranged from 20-53%. In contrast, the amount of above ground carbohydrate in 900 umol mol^{-1} CO_2 treatment as percentage of that in ambient CO_2 conditions ranged from 186% at 30 DAP down to only 118% at final harvest.

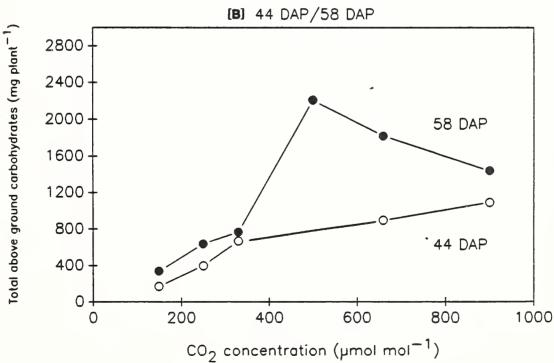
Figure 1A-D.

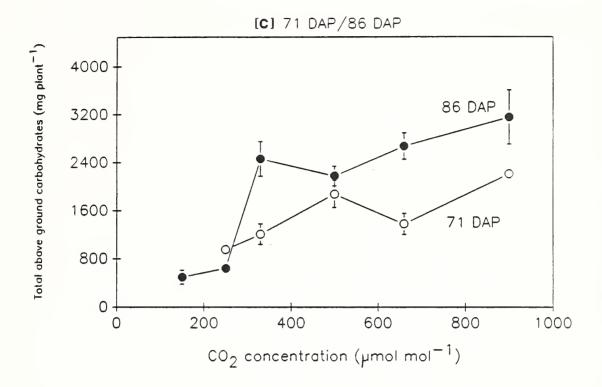
Seasonal total above ground (leaf blades, leaf sheath, culms, grain and chaff) nonstructural carbohydrate content in rice plants grown under a range of ${\rm CO_2}$ concentrations. Values are means \pm S.E. If no S.E. shown, value was smaller than the symbol except those at 30 and 58 DAP where one sample of 9 pooled plants were available for each treatment. DAP - days after planting.

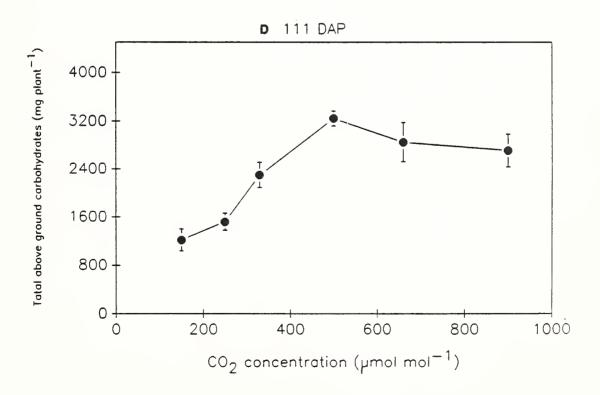
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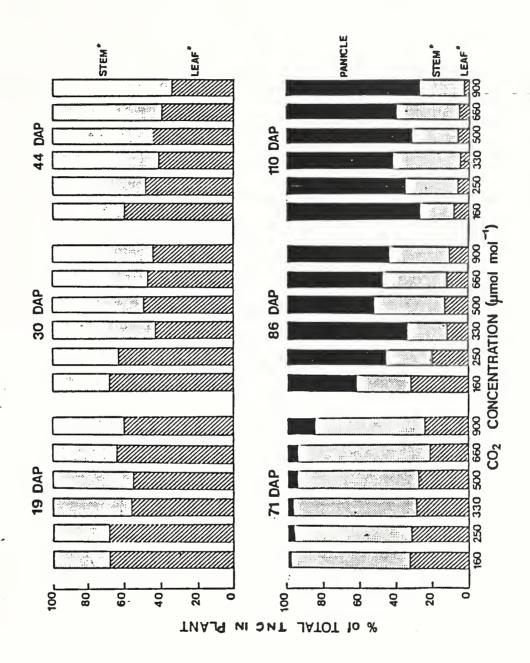
PARTITIONING OF TOTAL NONSTRUCTURAL CARBOHYDRATES BETWEEN PLANT PARTS

The changes in the percent TNC content in the various plants parts through the season are shown in Figure 2. Early in the season, most of TNC was contained in the leaf tissue $(57\% \pm 15 \text{ to } 68.5\% \pm 10\%)$ with no significant differences between the CO2 treatments. At 44 DAP, just before panicle initiation, 34% \pm 4 to 60.5% \pm 4.5 of the plant TNC was found in the leaf blades, with the percentage decreasing with increasing CO2. By 71 DAP, near the boot stage, most of the TNC was in the stem (56% \pm 4 to 73% \pm 15). Furthermore, the percentage of TNC in the panicle (grain and chaff) increased with increasing CO2. After 86 DAP (heading), there was still a large percentage of TNC in the leaf at CO2 concentrations less than 330 µmol mol⁻¹, with most of the TNC in the panicle. By the final harvest, the majority of the TNC was in the panicle with very little in the leaves in all the CO2 treatments, with little consistent trend between stem and panicle across the CO2 treatments.

Root TNC concentration was very low and made up only <1% of the total TNC in the plant. Therefore, root parameters are not considered further in this study.

Figure 2.

The effect of ${\rm CO}_2$ concentration on the partitioning of non-structural carbohydrates into various plants parts in rice through the season. DAP- days after planting.



TOTAL NONSTRUCTURAL CARBOHYDRATE CONCENTRATION

CO2 concentration had a significant effect on leaf TNC concentration over the growing season (P<0.0001, Tables 1 and 2). Seasonal means showed that TNC increased with increasing concentrations of CO_2 up to 330 µmol mol⁻¹ and enrichment above this levels had little affect on leaf TNC concentration (Table 1). Furthermore, there was a significant interaction between the CO2 regime and DAP (Table 2). The increases in TNC concentration in response to CO2 were most marked early in season (<58 DAP) where an average increase of 39% was measured between 160 and 900 µmol mol-1 (Fig. 3). However, the response declined by the final harvest (Fig. 3). By the end of the season the leaf TNC concentration of rice grown at or above 500 µmol mol⁻¹ was only 12% higher than that of plants grown at or under ambient CO2 concentration. The maximum TNC concentration in leaf blades occurred early in the season, during the tillering stage (Table 3).

The TNC concentration in the leaf sheath and culms was significantly affected by $\rm CO_2$ concentration (P<0.0001, Tables 1 and 2) and there was a significant interaction between $\rm CO_2$ concentration and DAP (P<0.0001, Table 2). The TNC concentration of leaf sheaths and culms increased as the $\rm CO_2$ concentration increased and leveled off at concentrations of $\rm CO_2$ above 330-500 μ mol mol⁻¹ (Fig. 4, Table 2). This response was seen throughout the season. The increase in TNC concentration of leaf sheath and culms averaged over

Table 1. Mean overall total non-structural carbohydrates concentration for all sampling dates.

CO ₂ (µmol mol)	<u>leaf TNC</u> * (mg g DW)	stem_TNC ⁺ (mg g ⁻¹ DW)	Panicle# (mg g ⁻¹ DW)
900	144 ^a	255 ^b	493 ^a
660	151 ^a	273 ^a	395 ^{bc}
500	152 ^a	239 ^b	416 ^{bc}
330	135 ^{ab}	252 ^{ab}	405 ^{bc} ·
250	120 ^b	196 ^C	362 ^C
160	118 ^b	157 ^d	431 ^b

Different letters denote significant differences (P < 0.05) between the ${\rm CO_2}$ treatments.

 $^{^{*}}$ leaf blades $^{+}$ leaf sheaths and culms $^{\#}$ grain and chaff

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Table 2. F-values from Anova of mixed two-factor experiment of total non-structural carbohydrate concentration throughout the growing season.

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model	<u>source</u>	<u>df</u>	<u>F-value</u>
0.91***	CO ₂ DAP CO ₂ * DAP	6 3 15	7*** 246*** 4***
0.75***	CO ₂ DAP CO ₂ * DAP	5 4 20	5.6*** 62*** 3***
0.86***	CO ₂ DAP CO ₂ * DAP	5 4 20	36*** 55*** 7***
	R ² 0.91*** 0.75***	0.91*** CO ₂ DAP CO ₂ * DAP 0.75*** CO ₂ DAP CO ₂ * DAP CO ₂ * DAP CO ₂ * DAP	0.91*** CO ₂ 6 DAP 3 CO ₂ * DAP 15 0.75*** CO ₂ 5 DAP 4 CO ₂ * DAP 20 0.86*** CO ₂ 5 DAP 4

^{***} P < 0.0001

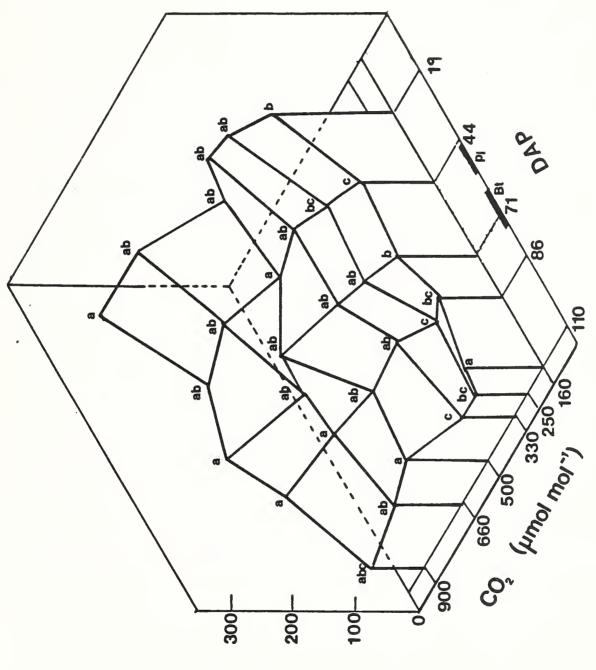
Figure 3

Total nonstructural carbohydrate concentration in leaf blades of rice plants grown under a range of ${\rm CO_2}$ concentrations through the season. DAP - days after planting. Different letters denote a significant difference (P<0.05) between ${\rm CO_2}$ treatments for a given DAP. Pi-panicle initiation stage; Bt-boot stage.

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TNC (mg g dw)

Table 3. Mean overall total non-structural carbohydrate concentration for all ${\rm CO}_2$ treatments over the season.

DAP	<pre>leaf TNC* (mg g⁻¹ DW)</pre>	stem TNC ⁺ (mg g ⁻¹ DW)	Panicle# (mg g ⁻¹ DW)
19	228 ^a	219 ^C	
44	165 ^b	262 ^b	
71	166 ^b	297ª	120 ^a
86	116 ^C	178 ^d	453 ^b
110	99 ^C	198 ^d	550 ^C

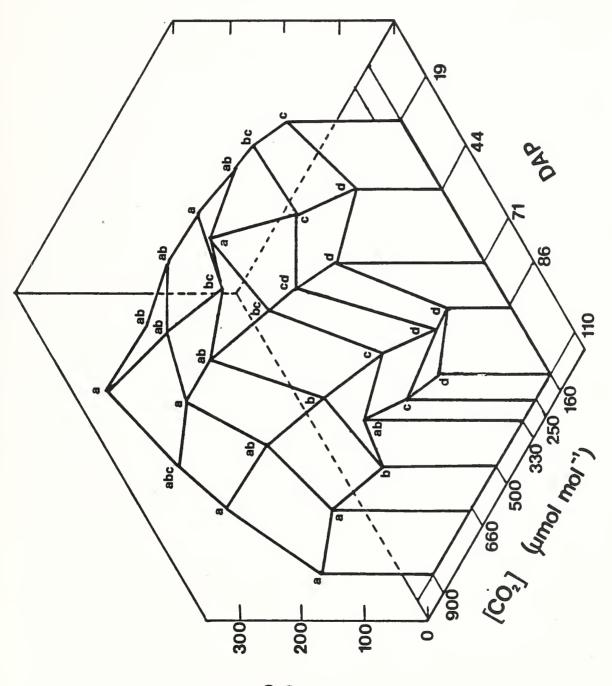
Different letters denote significant differences (P < 0.05) between the days after planting (DAP).

^{*} leaf blades; + leaf sheaths and culms

[#] grain and chaff except 110 DAP where value for grain only (chaff-135
mg g⁻¹DW at 110 DAP)

Figure 4

Total nonstructural carbohydrate concentration in culms and leaf sheaths of rice plants grown under a range of CO_2 concentrations through the season. DAP - days after planting. Different letters denote a significant difference (P<0.05) between CO_2 treatments for a given DAP.



TNC (mg g"d.w)

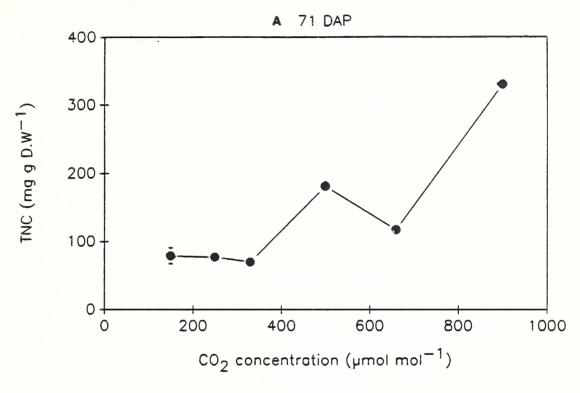
the season was 62% for an increase of CO_2 from 160 to 900 μ mol mol⁻¹ (Table 1). This was higher than that for leaf carbohydrate concentration which showed an increase of only 22% for the same increase in CO_2 concentration.

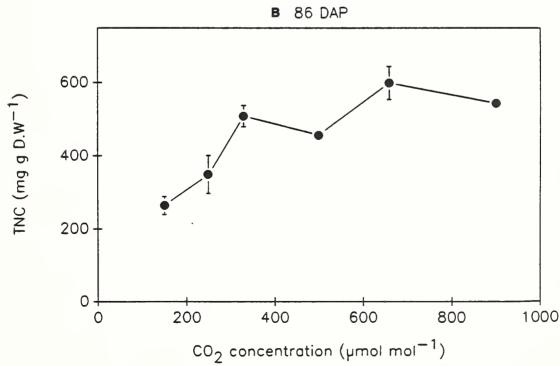
The TNC concentration reached a maximum in leaf sheaths and culms at 71 DAP, around the booting stage (Table 3). The TNC concentration in the leaf blades was of a similar order of magnitude to that in leaf sheaths and culms although the latter had the highest concentrations of carbohydrate, especially from the middle of the season onwards (Table 3).

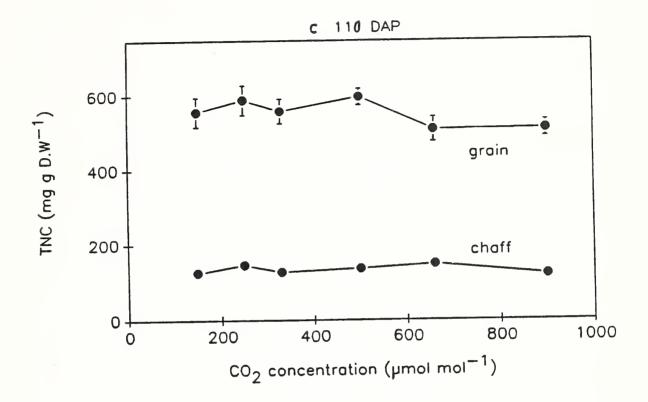
 ${\rm CO}_2$ concentration had a significant affect on TNC concentration of the panicle (grain and chaff) and there was a significant interaction with DAP (P<0.0001, Tables 1 and 2). The panicle TNC concentration was higher for plants grown under superambient ${\rm CO}_2$ conditions at or before 86 DAP (Figs. 5A and B). As the grain filling progressed, the TNC of the panicle increased in all ${\rm CO}_2$ treatments until grain TNC reached 550 \pm 15 mg g⁻¹ DW (without the chaff) at 110 DAP (Fig. 5C). Consequently, at final harvest, concentrations of carbohydrate in the grain and chaff were similar in all ${\rm CO}_2$ regimes (Fig. 5C).

Figure 5A-C

Seasonal changes in total nonstructural carbohydrate concentration in grain of rice plants grown under a range of ${\rm CO}_2$ regimes. Values are means \pm S.E. If no S.E. shown, value was smaller than the symbol. DAP - days after planting.







INPUT OF CARBOHYDRATES INTO THE PANICLE

The percentage of stored carbohydrates before heading contributed to grain fill is shown in Table 4. The % contribution increased as the $\rm CO_2$ concentration was increased up to 500 µmol mol⁻¹ and then decreased slightly as $\rm CO_2$ was raised above this level.

The rates of panicle filling were calculated as difference between panicle TNC concentration at various times. At 71 DAP, the rates increased as the CO₂ concentration was increased up to 500 µmol mol⁻¹ and leveled off above this (Table 5). Between 71 and 86 DAP rates of panicle TNC accumulation in the high CO₂ treatments had fallen. However, TNC accumulation rates were still lowest at the 160 µmol mol⁻¹. Betweem 86 and 108 DAP TNC accumulation rates at CO₂ concentrations above 330 were lower than before 86 DAP. In contrast, rates at concentrations of CO₂ below 330 were still at rates similar to earlier in the panicle filling stage.

CHEMICAL PARTITIONING OF ASSIMILATES AND ACCUMULATION OF SU-

Leaf samples were taken in the morning (am) and afternoon (pm) at 19 and 71 DAP. The PFD on these days and on the previous days to sampling are shown in Figure 6. The samples were used to analyze the partitioning of carbohydrate into sucrose, starch, fructose and fructans. The results for 19 and $\widehat{17}$ DAP are shown in Figures 7 and 8, respectively.

Table 4. The effect of CO₂ on the contribution of accumulated non-structural carbohydrates in vegetative plant parts at 58 days after planting to grain carbohydrate content at final harvest.

CO ₂ (µmol mol)	<pre>% contribution of accumulated TNC in vegetative parts to grain TNC content</pre>
900	36.0%
660	40.0%
500	54.0%
330	18.0%
250	11.0%
160	0.7% (18% ⁺)
contribution = 100	* TNC content in leaves and culms content at at 58 DAP
	TNC content in grain at maturity

⁺ at 71 DAP, when vegetative TNC content is at maximum for 160 µmol mol⁻¹ treatment.

Table 5 Rate of accumulation of TNC in panicles of rice grown under various ${\rm CO}_2$ concentrations.

	Panicle ⁺ TNC	accumulation r (mg CH ₂ O g ⁻¹ DW	ate d-1)
[co ₂]	71 DAP*	71-86 DAP	86-108 DAP
900	71	14	7
660	66	32 .	0
500	86	18	6
330	16	29	6
250	14	18	15
160	ND	12	17

^{*} determined as (pm-am) panicle TNC concentration

⁺ grain + chaff; ND- not determined

Figure 6.

Solar Photosynthetic photon flux densities (PFD) for the days on which samplings took place in the morning (<9:30hrs) and afternoon (>15:30hrs) and PFD for the previous days. DAP - days after planting.

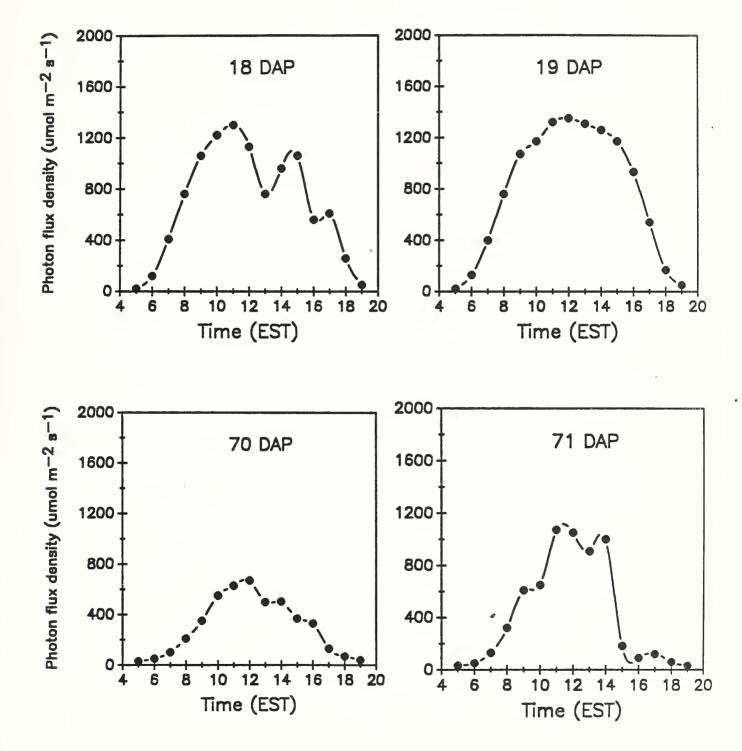
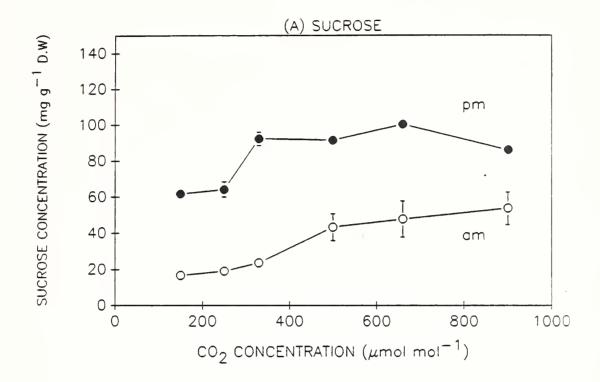
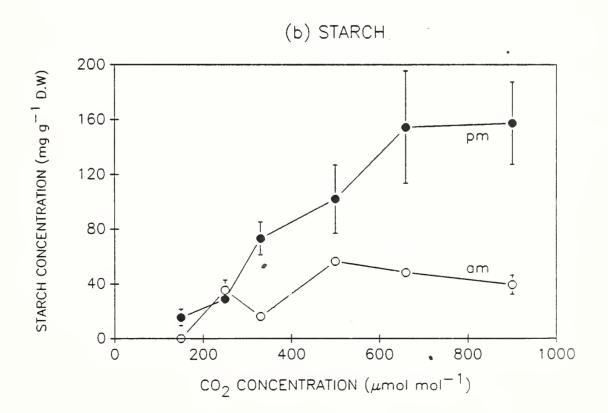
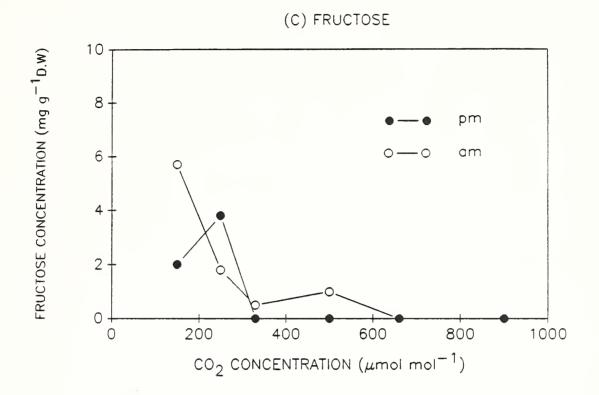


Figure 7.

Effect of CO_2 in the atmosphere on the concentration of (A) sucrose, (B) starch, (C) fructose and (D) fructans in rice leaf blades, in the morning and the afternoon, 19 days after planting. Values are means \pm S.E. If no S.E. shown, value was smaller than the symbol.







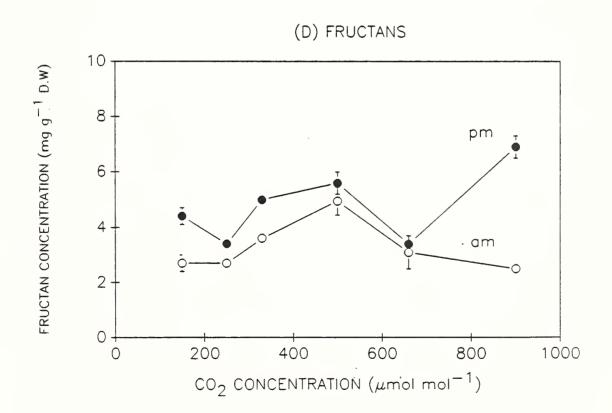


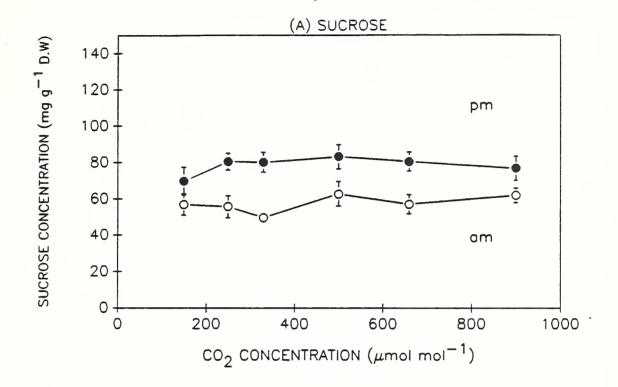
Figure 8

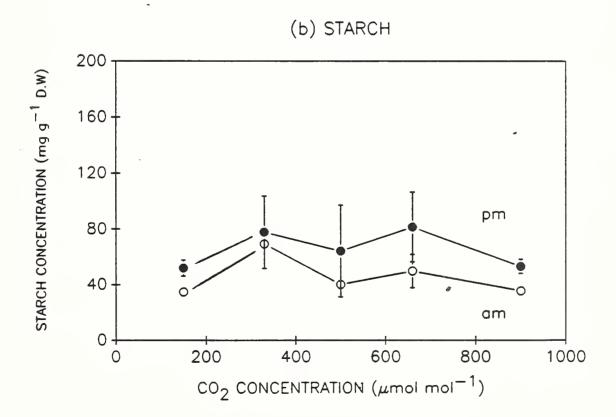
Effect of CO_2 in the atmosphere on the concentration of (A) sucrose, (B) starch, (C) fructose and (D) fructans in rice leaf blades, in the morning and the afternoon, 71 days after planting. Values are means \pm S.E. If no S.E. shown, value was smaller than the symbol.

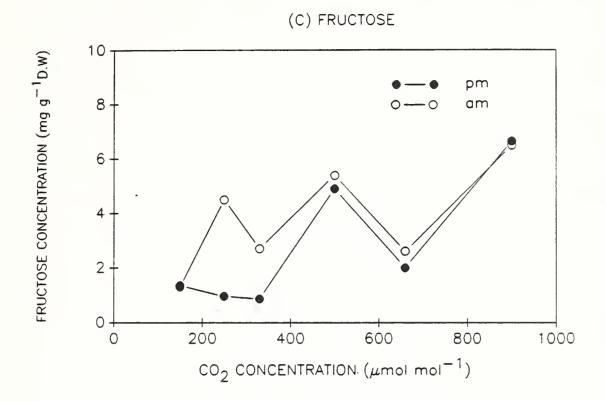
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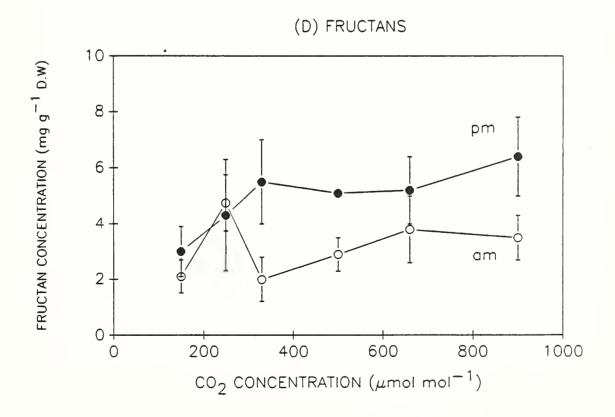
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Soluble sugars in leaves consisted largely of sucrose (1-10% of dry weight), while both glucose and fructose were present in low concentrations (<1% DW). High molecular weight storage was composed mainly of starch (2-16% DW), and fructans with a degree of polymerization >5 were low (<1% DW). For this study, fructans will refer to only those with a degree of polymerization of 5 or more.

Sucrose and starch concentration increased with rising CO_2 at 19 DAP ,however starch showed the greatest response (Figs. 7A and B). Across the 160 to 900 $\mu\mathrm{mol}$ mol^{-1} CO_2 treatments, pm sucrose concentration increased by 40% whereas starch levels increased 9 fold. Some leveling off of the response at the higher CO_2 concentrations was also measured. However, by 71 DAP, starch and sucrose levels were similar across all the CO_2 treatments (Figs. 8A and B).

The rates of starch accumulation (difference in starch concentration between am and pm samples) at 19 DAP increased as $\rm CO_2$ concentration increased (Fig. 7B), ranging from 18 \pm 6 mg starch $\rm g^{-1}$ day⁻¹ at 160 umol mol⁻¹ $\rm CO_2$ up to 120 \pm 30 mg starch $\rm g^{-1}$ day⁻¹ at 900 umol $\rm CO_2$ mol⁻¹. The rate of sucrose accumulation at 19 DAP ranged from 32 \pm 9 at 900 umol mol⁻¹ to 70 \pm 4 mg sucrose $\rm g^{-1}$ d⁻¹ at 330 µmol mol⁻¹. The percentage of the combined accumulation of starch and sucrose that was accounted for by starch accumulation increased with increasing $\rm CO_2$, ranging from 13 to 81% at 19 DAP.

The relationship between sucrose and starch concentration at 19 DAP (pm sample) is shown in Figure 9 and is described by the following equation:

$$R = 0.5 + (e^{(-0.0056[CO2] + 1.89)})....(1)$$

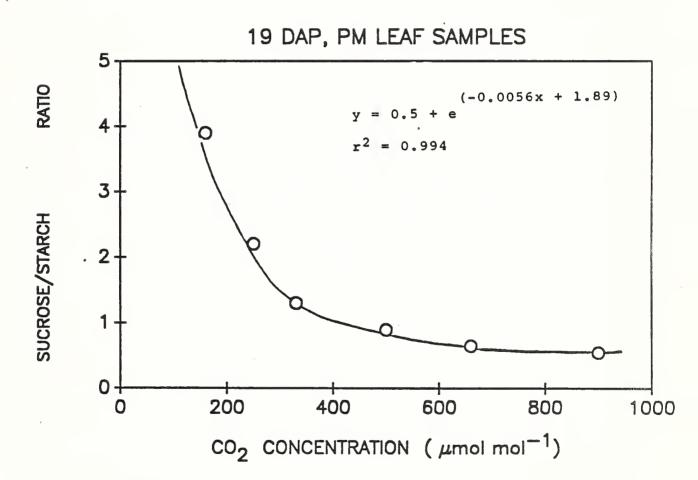
where R represents the ratio of sucrose to starch in the leaf. At the lowest CO₂ concentrations sucrose concentration was nearly 4 times that of starch and at the highest CO₂ treatment sucrose was present at only half the concentration of starch.

The rate of starch and sucrose accumulation at 71 DAP was much lower than that at 19 DAP (Fig. 8A and B). The rate of sucrose accumulation averaged 21 \pm 4 mg g⁻¹ DW day⁻¹ across the CO₂ treatments (Fig. 8A). The rate of starch accumulation was only significant at the lowest and highest CO₂ concentrations (19 \pm 5 mg g⁻¹ day⁻¹; Fig. 8B).

Fructose and fructan concentrations were low at both sampling dates, less than 1% DW (Figs. 7C,D and 8C,D). At 19 DAP the level of fructose dropped with increasing CO₂ (Fig. 7C) although no consistent trend was seen across the CO₂ concentrations, fructan levels were greatest at the highest CO₂ regime in the afternoon. The data were variable although a trend of increasing concentration of fructose and fructans with rising CO₂ can be observed at 71 DAP.

Figure 9.

The relationship between CO₂ concentration and the ratio of sucrose/starch concentration in rice plants at 19 DAP.



However, since levels of fructose and fructans were so low further interpretation is difficult due to variability.

In the leaf sheaths and culms at 19 DAP, starch made up to 30% of the dry weight, sucrose 16% and fructans 0.3%. At 71 DAP, starch also predominated with concentrations ranging from 20-40% DW. Sucrose levels were 6-8% DW and fructans averaged 1% DW.

LEAF EXPORT RATES AND STORAGE OF ASSIMILATES.

At 19 DAP, under ambient and superambient CO₂ concentrations, the percentage of carbohydrates fixed by photosynthesis that is exported from the leaf was greater than or equal to that stored (Table 6). Furthermore, export rates of carbohydrates from leaf blades were positively correlated with photosynthesis (i.e. carbohydrate production rate; Fig. 10). In contrast, at 71 DAP the percent stored was greater that exported in all CO₂ treatments except the 160 µmol mol⁻¹ treatment. Although export rates were highest at the higher CO₂ concentration, no linear relationship was observed between export rates and photosynthesis at 71 DAP (data not shown).

Table 6. The daily allocation of photosythetically fixed carbon to the processes of export and storage in the leaf blades of Oryza sativa L. in the light.

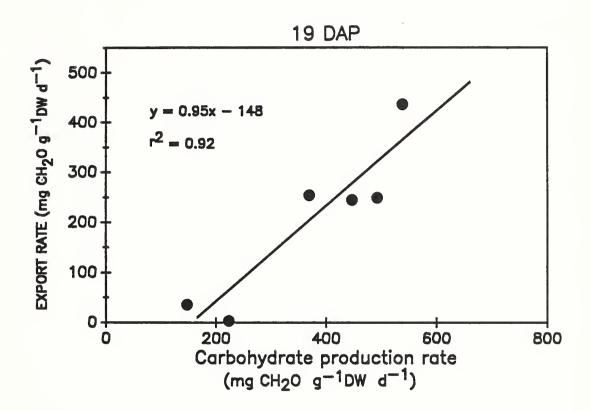
	§ of carboh	ydrate fixed by	net photosynth	nesis ⁺
[CO ₂]	TNC e	xport 71DAP	TNC s	storage 71DAP
900	50%	41%	50% -	59% 1
660	55%	13%	42% ↓	87% 个
500	81%	5%	19% ↓	95% ↑
330	69%	0%	31% ↓	100% ↑
250	1%	34%	9987	66% 1
160	24%	60%	76% 7	40%

⁺ adjusted for losses of carbon due to respiration.

¹⁴ higher or lower % storage than % export

Figure 10.

Relation between net carbohydrate production rate by photosynthesis and export rate of leaves at 19 DAP.



DISCUSSION

Increasing CO₂ up to 500 µmol mol⁻¹ in the atmosphere during growth resulted in an increase in the plant TNC content. CO2 enrichment above this concentration, however, did not result in any significant increase in plant TNC content. This paralleled the response of photosynthesis to CO2 concentration (see section IV). The increases in CO2 concentration and accompanying increases in photosynthesis, led to increases in both leaf TNC, culm TNC, leaf starch concentration, leaf sucrose concentration and export rate of carbohydrates out of leaf at the vegetative stages of development. Furthermore, the rate of leaf starch and sucrose accumulation increased with rising CO2 concentration. At anthesis, leaf and culm TNC concentration increased with increasing CO2 concentration, although there was no clear relationship between leaf export rate and CO2 treatment. The partitioning of carbon between carbohydrate pools of the leaf and export from the leaf are therefore functions of the changing sink demands associated with the stage of growth. In rice, elongating nodes along with culms and panicles can act as sinks for assimilates (Yoshida and Aln, 1968; Raskin and Kende, 1984).

Maximum carbohydrate content was reached in vegetative plant parts at heading (63-83 DAP). This result agrees with previous reports (see review by Yoshida, 1972). This is mainly due to the storage of carbohydrates in the culms of

the rice plants at this stage. In rice before flowering the culms accumulate carbohydrates and act as a sink (Yoshida and Aln, 1968). After flowering, most of the accumulated carbon moves into the panicles (Fig. 2).

Pre-anthesis assimilates stored in the leaf sheath and culms contribute substantially to grain filling (Fig. 2). With increasing CO2 treatment the contribution of stored assimilates to the grain increased (Table 4) as calculated by the method described by Yoshida and Ahn (1968). The percent contribution leveled off at 500 µmol mol⁻¹, the same concentration at which photosynthetic rates leveled off (see section IV). It appears that the contribution of stored assimilates may be related to the photosynthetic rate of the leaves. The contribution of this preheading storage has been shown to be variable, although it is usually around 20-40%, and is dependent on climatic conditions (Yoshida, 1972). The loss of stored carbohydrate from the vegetative parts during grain fill gives only a maximum estimates of contribution of stored carbohydrate to grain (Table 4) since the loss of stored carbohydrate during grain fill can also be partly due to respiration and some translocation to the roots. The carbohydrate in senesced leaves and aborted tillers was also not accounted for.

As well as affecting the contribution of previously stored carbohydrates to panicle filling, ${\rm CO_2}$ treatment also affected the rate of grain filling in rice plants. The maximum rate of grain filling was measured at the higher ${\rm CO_2}$

levels (>330 µmol mol⁻¹) early in the panicle production stage. The grain filling rate peaked between 71 and 86 DAP for 330 and 250 umol mol⁻¹ and between 86-108 DAP for the lowest CO₂ concentration. This probably reflects the later heading dates at the lower CO₂ regimes, grain carbohydrate input is usually greatest about 1 week after anthesis (Yoshida, 1972). Nevertheless, the rates of grain filling at low CO₂ concentrations never reached those at higher CO₂ concentrations.

In subambient CO2 treatments the small store of assimilates were still able to contribute to grain filling (Table 4) but at a much lower level than at higher CO2 concentrations. Current photosynthesis must have contributed the rest of carbohydrates needed for grain production. At maturity the concentration of carbohydrates in the grain was similar in all CO2 treatments, however, grain yield was higher at higher CO2 concentrations due to increase in number of panicles (see section III). The similar grain size (see section III) across the CO2 treatments indicates that the individual panicles were limited by capacity of sinks to utilize the extra C available. Despite this, the plants under CO2 enrichment still achieved greater total seed yield by increasing the number of panicles produced (see section III). The overall increase in yield that results from CO2 enrichment indicates that under current ambient CO2 concentrations rice panicle production is limited by the capacity of the

source leaves to provide photoassimilates. The total non-structural carbohydrate concentration in grain at final harvest were similar across all CO_2 treatments (553 \pm 15 mg g⁻¹ DW) and also in the percentage of total plant carbohydrate content in seed (66.8 \pm 2.6; Figure 2). The majority of the remainder of the carbohydrate was in the culm and leaf sheaths rather than in the leaf blades.

Rice yield has been related to the level of stored assimilates and the ability of the plant to translocate those assimilates to the panicle (Yoshida, 1972). Rice can respond to changing CO2 concentration by changing the priority of allocation of carbohydrates to storage or to export. Leaf carbon budgets for the light period revealed that plants grown under subambient CO2 condi tions, and restricted photosynthesis, had a priority for the storage of carbohydrates over the export of sucrose early in the season at the vegetative stage (Table 6). Furthermore, almost 80% of this stored carbohydrates was in the form of sucrose. As the CO2 concentration was increased the priority shifted more towards sucrose export than carbohydrates storage. The storage that did take place was mainly in the form of starch (up to 66%). In addition, the actual amount stored in rice at low CO2 concentrations was much lower than that stored in rice under high CO2 with higher photosynthetic rates. Under optimal conditions, some species can sequester up to 30% of their total dry weight in the form of storage carbohydrates (Smith, 1973). In rice, this was true at high CO2 concentra-

tions. CO₂ concentration therefore, not only alter the partitioning between storage and leaf export of carbohydrates (Table 6), but also the form in which the storage takes place (Fig. 8).

Carbohydrate export rate from the leaf in rice was found to be positively correlated to photosynthetic rate (mg $CH_2O g^{-1} DW d^{-1}$) early in the season. Maximum export of carbohydrates, which is assumed to be as sucrose, occurred at 500 umol mol^{-1} CO₂ when photosynthesis was at a maximum. By extrapolation in Figure 10, below photosynthetic rates of 151 mg CH_2O g^{-1} DW d^{-1} at 19 DAP, export would cease and presumably all the carbohydrate produced would go into storage. This would be at atmospheric CO2 concentrations below 160 umol mol⁻¹ for rice. This contrasts with soybean; in soybean it has been calculated that some export would still take place near zero photosynthetic rates (Fader and Koller, 1983; Huber et al, 1984). Low sink demand, due to small culms and low grain production, at concentrations of CO2 below 330 µmol mol⁻¹ may have led to the retention of carbohydrates in the vegetative parts and to the low export rates. Reduced sink demand induced in a number of ways such as restricted root growth and sink removal have been shown to increase the accumulation of carbohydrates in the source leaves (Ruftly and Huber, 1983; Robbins and Pharr, 1988).

A direct relationship between increases above a critical level in carbohydrate concentration and a decrease in

photosynthesis has been shown in wheat (Azcon-Bieto, 1983), soybean (Nafziger and Koller, 1976), cucumber (Pharr et al, 1985) and P. maximum (Ariovich and Cresswell, 1983). The accumulation of starch within chloroplast can be accompanied by damage and disorientation of grana and thylakoids (Carmi and Shoner, 1979: Wildman et al, 1980) which may result in reduced photosynthetic rates (Troughton, 1975).

In rice, starch accumulated during the photoperiod mainly early on in the season at superambient CO2 concentrations, accounting for on average 19% of the production rate of carbohydrates by photosynthesis. This level dropped to an average of 7% at subambient CO2 concentrations. At heading, daytime accumulation of starch was small, significant only at 160 and 900 µmol mol⁻¹ CO₂ concentrations, al though it was about 24% of the carbohydrates produced by photosynthesis in these treatments. Others have measured increases in starch content with increasing CO2 (Thomas et al, 1975; Finn and Brun, 1982; Peet et al, 1986; Allen et al, 1988). Allen et al (1988) found that nearly all of the extra carbon fixed as a result of CO2 enrichment in soybean was partitioned into starch. However, decreases in photosynthetic rates were not observed at the higher CO2 concentrations in their studies. Similarly, in rice there is no evidence that the accumulation of starch in the leaf was detrimental to the photosynthetic rate of the plant. In plants grown at higher CO2 concentration, which are most likely to suffer from excessive leaf starch accumulation, the priority was in fact for



export rather than for storage, so avoiding this situation. Furthermore, the leveling off of the photosynthetic rate at CO₂ concentrations above 500 µmol mol⁻¹ were apparent at both stages of development (see section IV) although starch accumulation at the later stage of development was similar across the CO₂ concentrations. Consequently, it seems unlikely that leaf starch accumulation was the primary cause of the lack of response of photosynthesis above 500 µmol mol⁻¹. Another part of this study suggests that the cause may involve a reduction in the activity and content of the enzyme responsible for the fixation of CO₂, ribulose 1,5-bisphosphate carboxylase (see section VI).

There was a general lack of accumulation of glucose, fructose and fructans, however they could be turning over substantially, especially the fructans (Farrar and Farrar, 1985). It is acknowledged in this study that examining total tissue concentration of these compounds is limited in that various pools of storage and transport compounds exist for sucrose (Farrar and Farrar, 1985) and that turnover of these pools takes place.

Most of the carbohydrate stored in many crop species, such as soybean, is in the form of starch (Allen et al, 1988; Mauney et al, 1979) while sugars are usually present in low concentrations. However, for rice, the sucrose pool was often as large as that of starch pool (Figs. 7 and 9) and greater under conditions of low CO₂. Furthermore, the

sucrose concentration increased in rice leaves in response to increasing CO₂ concentration, whereas in soybean the sucrose pool did not respond to increasing CO₂ concentration (Allen et al, 1988). In contrast with our results for rice, increasing photosynthesis during vegetative growth resulted mainly in an increase in starch levels (Mauney et al 1979; Nafziger and Koller, 1976; Allen et al, 1988). When pods developed, however, an increase in photosynthesis is accompanied by a decrease in the concentration of starch and an increase in that of sucrose. Again, at different growth stages and sink demands the shifts in carbon flow can change.

Sucrose plays an important role as a major end product of photosynthesis, as a major form of translocated carbon and also as a storage sugar. Sucrose synthesis in the cytosol is the main result of photosynthesis (Farrar and Farrar, 1985). There are 3 major fates of this sucrose; storage, translocation and conversion to fructans. In plants that mainly accumulate starch such as soybeans, (Allen et al, 1988) most of the cytosolic sucrose is translocated and starch degradation during the dark period provides substrate for sucrose synthesis. In species that accumulate mainly sucrose, such as barley (Farrar and Farrar, 1985), this is stored (probably in the vacuole) during the light and mobilized in the dark to maintain cytosolic sucrose and translocation.

The formation of sucrose and starch are interdependent as they compete for the pool of triose phosphates produced

by the calvin cycle. It appears that sucrose formation in the cytoplasm and starch accumulation in chloroplast are reciprocally related, especially at early stages of development (Huber and Israel, 1982 and Fig. 8). Furthermore factors that regulate starch synthesis also regulate sucrose synthesis. Kerr et al (1984) suggested that starch accumulation is controlled, in part, by activity of sucrose phosphate synthase (SPS). In rice, at low CO₂ concentration sucrose predominates and at high CO₂ starch predominates. This may reflect changes in SPS and requires further investigation. The ratio of sucrose to starch appeared to reach a threshold level as the CO₂ concentration increased at both stages of development examined (0.55 at 19 DAP and 1.21 at 71 DAP at pm). This may be genetically predetermined.

Some implications, as shown in this study, for CO₂ enriched atmospheres of the future include the possibility that the vegetative period will be shorter (see Section II) which could have critical bearing on how rice will respond to periods of stress. This is due to the fact that at this time storage of carbohydrate for use in grain filling is considerable. If the vegetative period is shorten before development of a stress, for example water stress, which results in a reduction in photosynthesis and synthesis of carbohydrate, then yield could suffer. It appears more likely, however, that sufficient carbohydrate is stored in the shorter time due to the increase in the supply of CO₂ and

higher photosynthetic rates to allow the plant to fulfill the requirement for grain filling and withstand a drop in the optimum conditions for C fixation or distribution later in the season. The predicted increase in CO₂ concentration over the next century would probably result in an increase in the concentration of carbohydrate in the vegetative plant part but not in the grain, although total grain yield would improve due to an increase in the number of panicles and available carbohydrate to fill the extra grain. However, increasing CO₂ would not affect the partitioning of available C among the plant parts at maturity.

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SECTION VIII

Genetically Altered Cyanobacteria as Nitrogen Fertilizer Supplier for Growth of Rice

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VIII. Genetically Altered Cyanobacteria as Nitrogen Fertilizer Supplier for Growth of Rice Introduction

Nitrogen, the most abundant gas in the atmosphere, is the element most often limiting for plant growth and reproduction. Unfortunately, plants cannot assimilate atmospheric nitrogen directly. They need a combined form such as nitrate or ammonia. In agricultural environments this requirement is provided as nitrogen fertilizer or green manure. Recent yield increases in cereal production have been achieved by higher fertilizer use in conjunction with improved high-yielding crop varieties that respond to high nitrogen fertilizer application (5, 20). The limited supply and increasing cost of fossil fuels used for producing fertilizer and unpredictable delivery systems make it imperative that alternate low cost, low technology sources of nitrogen fertilizer be developed to maximize crop production especially in developing countries (17).

Cyanobacteria, formerly called blue-green algae, make a valuable contribution to the fertility of many soils (21, 31). They are ubiquitous and grow over a wide climatic range (9). These photosynthetic organisms have the ability to reduce, or fix, atmospheric nitrogen to ammonia. Many genera can fix considerable quantities of dinitrogen, ranging from 1 kg/ha.yr in the Antarctic to around 30 kg/ha.yr in temperate, arable soils to nearly 70 kg/ha.yr in tropical rice paddy fields. It has been suggested that nitrogen fixation by cyanobacteria

is responsible for continued rice production, over extended periods of time, where little or no fertilizer is added to the soil.

Free-living, nitrogen-fixing organisms do not excrete ammonia into the plant environment. In most nitrogen-fixing organisms, ammonia production by the enzyme complex nitrogenase is tightly coupled to utilization within the organism and the fixed nitrogen is not immediately available for crop growth. The plant benefits only after the bacteria die and the nitrogen is released by mineralization (8, 15, If one of these free-living organisms could be 30). genetically altered to export the nitrogenase-produced ammonia into the environment, then this might be a feasible mechanism for reducing nitrogen stress in crops. Mutant cyanobacterial strains that are not able to assimilate the reduced nitrogen into organic compounds can be generated and isolated by appropriate selection conditions. These mutants, used as soil inoculants, can potentially provide a substantial amount of nitrogen fertilizer for crop growth.

Ammonia Assimilation

The ammonia produced by nitrogenase is assimlated by the enzymes glutamine synthetase and glutamate synthase (24). Inhibition of glutamine synthetase by a glutamate analog, L-methionine-D,L-sulfoxi-mine (MSX) blocks the assimilation of ammonia and it is excreted into the medium. This observation was extended to include all the free-living, nitrogen-fixing organisms and in all the organisms studied so far, addition

of MSX or other glutamate analogs led to excretion of ammonia This block in the assimilation of ammonia into the medium. includes the utilization of exogenous Inhibition of growth by MSX can be readily overcome by adding glutamine to the medium as a nitrogen source. Genetic studies have confirmed these observations. Mutant strains lacking active ammonia assimilation enzymes were initially isolated from Klebsiella pneumoniae strain M5A1 (23). These mutant strains excreted the ammonia produced from dinitrogen as well as from nitrate and also failed to assimilate externally added ammonia. This block in ammonia assimilation led to glutamate or glutamine auxotrophy, depending on the location of the mutation (25). The inability to assimilate the ammonia from the medium produced a nitrogen deficiency, even in the presence of high concentrations of ammonia in the medium. Since N-limitation generally leads to derepression nitrogenase synthesis, ammonia assimilation-defective mutants as well as wild type cultures supplemented with MSX, derepressed nitrogenase synthesis in a medium containing ammonia. This property allowed the cell to produce and excrete ammonia in a medium already rich in ammonia. studies now include several nitrogen-fixing bacteria (10, 18, 22, 26, 27, 33, 34).

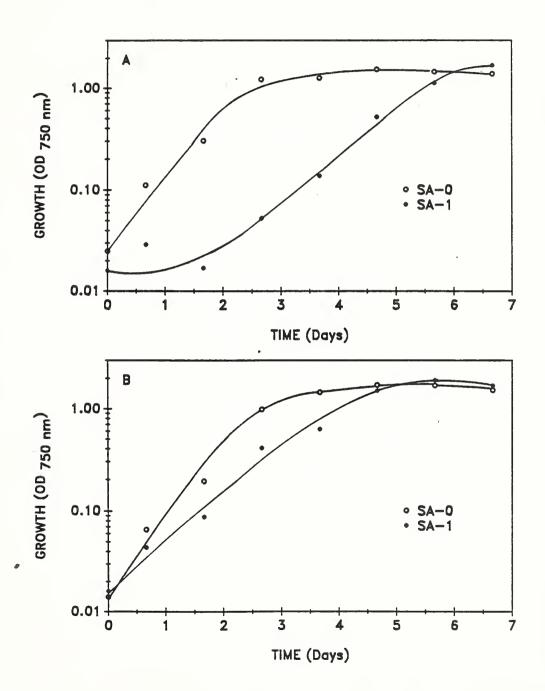
Before the potential use of ammonia-excreting mutant strains in crop production can be achieved, two aspects of nitrogen fixation need to be considered. The first constraint is the energy cost of nitrogen fixation. Nitrogenase consumes

at least 12 moles of ATP for every mole of dinitrogen reduced to ammonia (36). This value is much higher in a nitrogenfixing cell which includes, in addition to nitrogen fixation, the basic metabolic processes required to supply reductant and energy for nitrogenase activity and the concomitant loss of hydrogen gas during nitrogenase activity. A minimum value of 24 ATP/dinitrogen has been reported for nitrogen-fixing mutant strains of K. pneumoniae which excrete ammonia into the medium The second constraint is that these mutant strains require organic nitrogen compounds for growth and, from an economic point of view, maintaining such microorganisms outside the confines of the laboratory would be costly although many of the nitrogen-fixing heterotrophic bacteria have been isolated from the roots of crop plants (32). Before these mutant strains can be tested for their abilty to supply nitrogen to cereals, both these constraints need to be addressed. Besides these requirements, an appreciation of how ammonia regulates nitrogenase biosynthesis and production is also needed (11).

By appropriate selection conditions, conditional mutant strains of cyanobacteria can be readily isolated. These mutant strains, which utilize sunlight and water for nitrogen fixation, do not have a requirement for organic nitrogen compounds (27, 28). Similar mutant strains of the filamentous, heterocystous cyanobacterium Anabaena variabilis and Nostoc muscorum, which excrete ammonia into the environment, have been described (26, 27). These mutants can

Figure 1. Growth of <u>Anabaena variabliis</u> parent, strain SA-0 and mutant, strain SA-1, in the presence of N_2 (A) or NH_4^+ (B) as the nitrogen soure. Cultures grown at 30°C in A/2 medium (27) containing fructose (10mM) and HEPES (10mM). Concentration of NH_4^+ was 3 mM. Growth was monitored as increase in optical density at 750 nm.

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potentially serve as nitrogen fetilizer "factories" to support the growth of cereal and forage plants in nitrogen deficient environments. An ammonia-excreting mutant of \underline{A} . variabilis, strain SA-1, was found to supply ammonia for the growth of rice in glasshouse experiments (18). The results of additional experiments on the potential agronomic use of this organism and the mechanism of the ammonia excretion are presented in this report.

Excretion of Ammonia by Mutant Strain SA-1

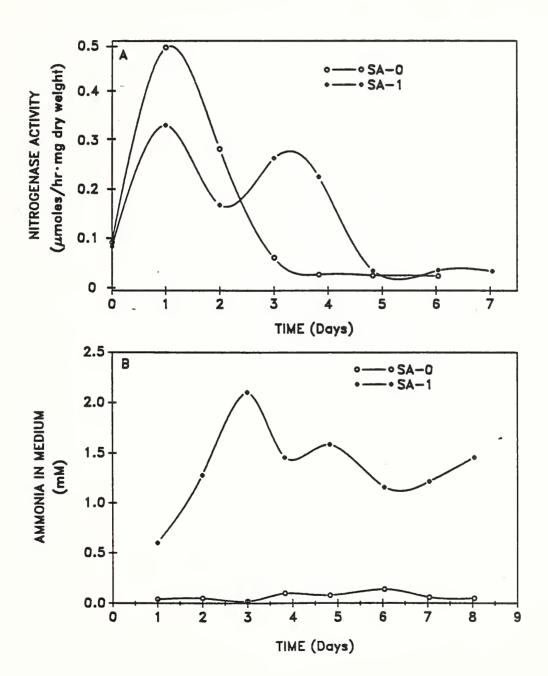
Although strain SA-1 exhibits substantial nitrogenase activity, this strain grew slowly, and failed to produce a colony on an appropriate solid media with dinitrogen as the sole nitrogen source. However, in liquid cultures, strain SA-1 grew at the expense of dinitrogen after a lag period of about 1.5 days. The generation time for the mutant strain in a fructose-supplemented medium under photoheterotrophic growth conditions at 30°C was about 16 hours with dinotrogen as the only nitrogen source as compared to the generation time of 10 hours for the parent strain (SA-0) in the same medium (Figure 1A). This lag in the growth of strain SA-1 was not observed if ammonia (3mM) served as a nitrogen source (Figure 1B). The generation times for the parent and mutant cyanobacteria, in the presence of ammonia, were 10 and 15 hours, respectively. It is probable that the lag in growth observed in liquid cultures, where dinotrogen is the sole nitrogen source, corresponds to accumulation of ammonia produced and excreted into the medium and growth occurs at the expense of the

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ammonia in the medium only after it has reached an adequate concentration. Both cultures produced nintrogenase activity (Figure 2A) during early stages of growth in N-free medium and this activity declined with the cessation of growth. mutant strain is capable of excreting the ammonia produced by the nitrogenase into the environment (Figure 2B). During the first day of incubation, strain SA-1 produced by the nitrogenase into the environment (Figure 2B). During the first day of incubation, strain SA-1 produced about 0.55 moles of ammonia per ml of culture. The parent strain, under these conditions, produced little or no ammonia. Growth of the strain coincided with the mutant presence of concentrations of ammonia in the medium. The rate of ammonia production by the mutant strain, during the lag period, was about 0.6 moles/hr.mg dry weight. Similar results were also obtained when the cultures were grown under photoautotrophic conditions with carbon dioxide as the sole carbon source.

The free-living batch cultures produced ammonia only during the growth of the organism. Upon cessation of growth, the rate of ammonia production declined to a low level. However, if the filaments were immobilized in carrageenan beads and consistently supplied with a fresh medium, ammonia production could be sustained for long periods. The amount of ammonia produced and accumulated by the mutant increased linearly with time (Fig. 3A). Nitrogenase acitivity of the filaments in the beads started to increase after reaching a low value at about 10-15 days after the start of the

Figure 2. Levels of nitrogenase activity (A) and $\mathrm{NH_4}^+$ production (B) by strains SA-0 and SA-1. Cultures were grown at 30°C in A/2 medium containing fructose (10 mM) and HEPES (10 mM) (27). Dinitrogenase activity of the samples were measured as acetylened reduction activity. The amount of $\mathrm{NH_4}^+$ present in the culture medium was determined using Nessler's reagent using procedures described before (2).

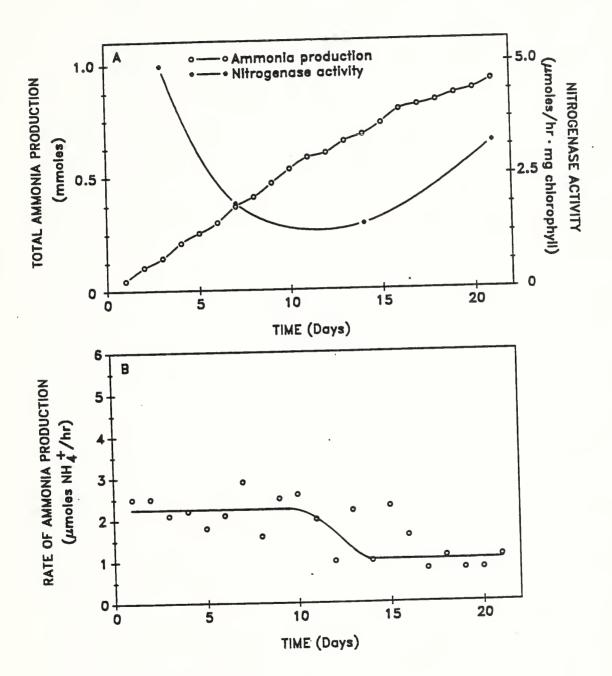


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Figure 3.

Ammonia production (A) and rate of ammonia production (B) by immobilized cells variabilis strain SA-1. Cells obtained from (2mM) - grown cultures limiting NH, were in K-carrageenan immobilized (19).The immobilized cells in beads of approximately 2 mm in diameter were placed in a bioreactor (35 cm 2.1 cm diameter) and A/2 medium supplemented with KCl (50 mM) and low phosphate (0.1 mM) was pumped through the bioreactor at a constant rate of 8.5 ml/hr. The average wet weight of the beads was 74 mg and the total weight of the beads in the bioreactor was 9.24 grams. The average chlorophyll content of the beads was 0.48 g/bead. The cells used for immobilization had a nitrogenase activity of 6.74 moles ethylene formed/hr.mq chlorophyll. Immediately after immobilization, nitrogenase activity of the filaments in the beads was below the detection limits of the acetylene reduction assay. Air was passed through the bioreactor to supply dinitrogen and ambient CO, served as the source of carbon.

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experiment. The rate of ammonia production was maintained between 1.5 and 2.5 moles/hr in the bioreactor (Figure 3B). In a separate experiment, this rate was maintained at a higher level of about 5 to 6 mol moles/hr for over 30 days when the flow rate of fresh medium was increased by five fold. There was a corresponding increase in the total ammonia produced by the cells in the bioreactor under these conditions. The possibility exists, that under appropriate conditions, the mutant strain can produce ammonia at a steady rate for several weeks.

The fact that strain SA-1 was excreting ammonia from dinotrogen and not releasing organic nitrogen compounds was confirmed by bioassay. K. pneumoniae strain SK-24 lacks the ammonia assimilation enzymes glutamate synthase and glutamate dehydrogenase and is a glutamate auxotroph (23). This strain utilizes amino acids, such as glutamate, glutamine, aspartate and others, but not ammonia for growth. When strain SK-24 was co-cultured with the ammonia-excreting A. variabilis strain SA-1, in a sucrose-supplemented medium, strain SK-24 did not grow. If strain SA-1 had excreted organic nitrogen compounds, such as amino acids, then the growth of K. pneumoniae strain SK-24 would have been detected. Strain SA-1 also supplies nitrogen and supports the growth of non-nitrogen-fixing, ammonia-assimilating organisms, such as Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes and the aquatic plant Lemna minor in co-culture.

These experiments confirm that strain SA-1 is a

conditional auxotroph (for glutamate) only when the ammonia concentration is maintained at a low level, either by diffusion, as occurred when the organism was cultured on the surface of a solid medium, or a coculture with ammonia-assimilating organisms. The defect in the assimilation ammonia, when present in low concentrations, suggests that one or both of the "ammonia-assimilation" enzymes are altered. Since strain SA-1 was isolated as an MSX-resistant mutant, properties of its glutamine synthetase were evaluated. Glutamine synthetase from the mutant was resistant to MSX and inhibited by only 10% even at an MSX concentration as high as 0.2 mM. The enzyme from the parent was completely inhibited by about 25-30 M MSX.

Glutamine Synthetase

To determine the kinetic properties of glutamine synthetase, the enzymes from the parent (SA-0) and the mutant (SA-1) strains were purified to homogeneity. The apparent Km values for different substrates were higher for the mutant enzyme as compared with the parent enzyme (Table 1). At pH 7.5, the optimum for the parent enzyme, the apparent Km values for the mutant enzyme were higher by 93-fold for ammonia, 10-fold for glutamate and 1.6-fold for ATP. Although the apparent Km values were higher for the enzyme from strain SA-1, the rate of substrate turnover by the two enzymes were similar (2,985 and 2,263 moles/min per mole of the two enzymes from SA-0 and SA-1, respectively). At pH 6.8, the optimum pH value for the mutant enzyme from the mutant, the difference

Table 1.	Kinetic Paramet Strains of SA-0	Kinetic Parameters of Glutamine Synthetase from <u>Anabaena variabilis</u> Strains of SA-0 and SA-1.	etase from <u>Ana</u> l	oaena variabilis
Strain		Apparent Km (mM)		Molecular
	NH ₄ +	Glutamate	ATP	Activity
At pH 7.5	7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7			
SA-0	0.015	1.74	0.13	2,985
SA-1	1.400	16.78	0.21	2,263
At pH 6.8				
SA-0	0.040	1.45	0.10	2,450
SA-1	12.400	9.20	0.17	3,533

Molecular activity is moles per min per mole of enzyme.

in the apparent Km for ammonia was increased to 310 fold. The Km values for other substrates were not significantly altered at pH 6.8 as compared to the values obtained at pH 7.5. These results suggest that strain SA-1's inability to utilize low concentrations of ammonia is a consequence of the change in the affinity of the enzyme for its substrates.

Although strains SA-0 and SA-1 differ in ability to assimilate ammonia they are similar in most other phenotypic characteristics. However, other phenotypic differences have been observed. Strain SA-1 appears able to grow at higher temperatures than strain SA-0 (data not presented) and has, generally, a darker green color when grown in liquid culture. This last observation prompted an examination of their phycobiliproteins. These accessory pigments are more than two times greater in SA-1 than SA-0 on a dry weight basis and almost 6 times greater on a chlorophyll basis (Table 2). The difference in phycobiliproteins between the strains suggests an increase in the ability to absorb and transfer light energy to chlorophyll by strain SA-1, however, differences in photosynthetic ability between the two strains have not yet been investigated.

Rice: Cyanobacterial Interactions

The carbon dioxide concentration in the atmosphere has increased from about 315 ppm in 1958 to over 345 ppm in 1985 and will double soon due to the continued burning of fossil fuels (3, 4, 7). Photoysnthesis and stomatal physiology are directly affected by atmospheric carbon dioxide concentration

Concentration of Phycobiliproteins in Strains SA-0 and SA-1ª. Table 2.

Strain	mg/g dry wt.	mg/g chlorophyll
SA-0	21.6	1,718
SA-1	45.3	6,216

Three day old cultures, grown at room temperture with 10 mM fructose, 3 mM ammonia, 10 mM HEPES buffer and 3 mM phosphate were used in this experiment. ಹ

while many other plant processes are indirectly affected. Elevated carbon dioxide levels have been shown to increase growth and yield of rice (6) and have increased the nitrogen fixation capacity of symbiotic systems (12, 35). Hardy and Havelka (13) report dramatic increases in nitrogen fixation by soybean (Glycine max L. Merr.) during carbon dioxide enrichment. These substantial effects were observed in as little as seven days and the amount of nitrogen fixed was increased from 75 to 425 kg/ha. The increase in nitrogen fixation produced by carbon dioxide enrichment was attributed to an increased net production of photosynthate. However, relatively little is known about the efects of CO, on the nitrogen-fixing capacity of cyanobacteria growing association with rice. As rice provides half the diet of 1.6 billion people and contributes between a fourth and a half of the diet for another 400 million (29) it is important to determine the responses of these microorganisms to changing levels of carbon dioxide. The objectives of this part of our study were to determine the effect of inoculation with nitrogen-fixing strains of cyanobacteria on the yield responses of paddy-grown rice and, further, to compare these responses under season-long exposure to ambient superambient levels of carbon dioxide.

Our tests on this association of strains SA-0 and SA-1 with rice have been made in the glasshouse (1, 18) and in controlled-environment plant growth chambers located on the Irrigation Research and Education Park at the University of

Florida in Gainesville, Florida. These chambers were 2 m x 1 m in ground area and 1.5 m in height. They have been described in detail by Jones et al. (16). During this experiment the units were adjusted to maintain dry bulb and dewpoint temperatures of 31° and 18° respectively. carbon dioxide concentration was maintained at either 330 (ambient treatment), 660 or 900 (superambient) ppm. A CO, injection system was used to replace the carbon dioxide taken up by the plants and cyanobacteria during photosynthesis. Paddy flood water depth was maintained at 5 cm above the soil surface and water temperture was controlled to 27°C. experiment was conducted during 1987 with a planting date of 22 January and a harvest date of June 1. The photoperiod was extended from 1700 to 2400 Eastern Standard Time until 2 March with four supplemental incandescent 75-watt lights over each chamber. Plants received an average total solar radiation of 14.17 MJ/m² per day. Rice plants (Oryza sativa L. cv. IR-30) were grown in large glass tubes (7 cm dia. x 50 cm ht.) filled to 8 cm of the rim with Chandler fine sand. The tubes were arranged in a completely randomized design with replications and placed into exiting rows (17.8 cm apart). These glass tubes prevented the chamber paddy water from contaminating the bacteria used in this study. Shades were maintained at canopy height along the outside of the chamber to simulate the light conditions existing in a field canopy. Prior to flooding, each chamber was fertilized with P and K, both at rates of 11 g/m^2 . Nitrogen was not applied to the

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inoculated plants grown in the glass tubes. The plants were inoculated with either strain SA-0 or strain SA-1, on February 6 and reinoculated on March 10, and received 9.6 mg dry weight per plant at each date. The inoculum was prepared and applied as described previously (18). At harvest, the plants were fractionated into roots, shoots, and grain, and then dried to a constant weight at 105°C. Nitrogen analysis was done by Kjeldahl procedure and the ammonia determined by semiautomated colorimetry.

Under these conditions, which span large superambient CO, concentrations, we found small changes in plant biomass in response to increasing carbon dioxide levels (Table 3). Plant dry weight increases were directly proportional to the elevated carbon dioxide concentrations. The increases were independent of inoculation protocol or yield component (shoot, root or grain) but did not differ significantly between CO. treatments. These results are consistant with other studies on rice (14; J.T. Baker, personal communication). By the end of the growing season the above-ground biomass of the 900 ppm treatment was only 1.18 times, on average, that of the 330 ppm treatment. The partitioning of biomass between root and shoot was not affected by increasing CO, concentration (Table 3), although increases in root:total plant dry weight ratios with CO, enrichment have been reported for rice (14). The response to carbon dioxide levels by grain yield of the inoculated plants is very similar to urea fertilized (170 kg urea/ha), non-inoculated plants grown under identical conditions (J.T.



Effect of Inoculation with Strains SA-0 and SA-1 on Growth and Development of Rice. Table 3.

		Shoot	Root	Grain	Root/shoot
Strain ${\rm CO}_2^+$	co ₂ +	Dry Wt. #	Dry Wt.	Dry Wt.	Ratio
SA-0	0000	1.96 ± 0.20a [§]	0.84 ± 0.12a	0.72 ± 0.17a	0.30 ± 0.01
SA-1		$2.71 \pm 0.39b$	1.09 ± 0.19b	1.29 ± 0.42bc	0.29 ± 0.02
SA-0	099	2.19 ± 0.38a	0.96 ± 0.27ab	0.87 ± 0.28ab	0.31 ± 0.04
SA-1		2.82 ± 0.19b	1.22 ± 0.17bc	1.54 ± 0.53c	0.28 ± 0.03
SA-0	000	2.24 ± 0.29a	1.04 ± 0.27ab	1.05 ± 0.36ab	0.31 ± 0.03
SA-1		2.96 ± 0.24b	1.35 ± 0.26c	1.64 ± 0.35c	0.29 ± 0.02

+ Carbon dioxide concentration in the atmosphere of the chambers.

Expressed as grams per plant at maturity ± SD.

§ Values followed by the same letter in each column are not significantly different by Duncan's multiple range test (P=0.05).

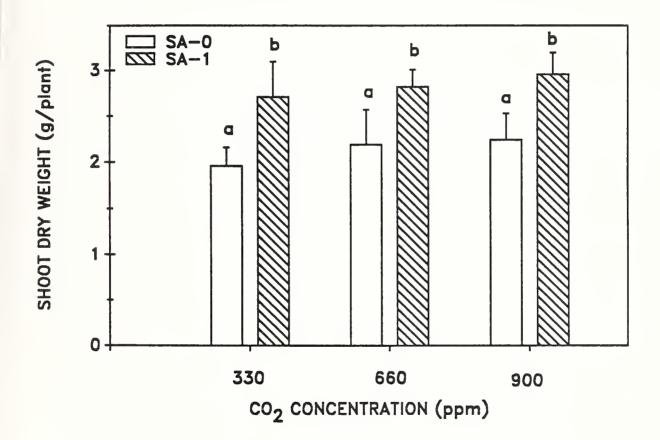
Baker, personal communication). The number of panicles per plant was low, only 64% of the urea fertilized plants, and showed a slight, but not significant, increase in number with increasing CO₂ enrichment. Imai et al. (14) have reported small but significant increases in number with increasing CO₂ enrichment. However, they attributed the majority of the yield response to an increase in the number of seeds per plant which resulted from an increase in the number of panicles per plant.

Inoculation of rice with strain SA-1 increased biomass production of all yield components when compared to plants inoculated with strain SA-0 (Table 3 and Figures 4, 5, 6, and The increases due to strain SA-1 were independent of carbon dioxide level, and were significant (P = 0.05) at each carbon dioxide concentration and for every yield component investigated except for roots grown at the 660 ppm CO, level. Inoculation with the ammonia-excreting strain increased panicle number (data not shown) by 21% and grain yield by more than 70%, on average, over plants inoculated with the parent strain. Above ground biomass (shoots, Figure 4, and grain, Figure 6) of plants inoculated with strain SA-1 was almost 73% that of uninoculated, urea fertilized plants at the 330 ppm CO, level, however, the percentage declined to 58% at both superambient CO, levels (Section II). This suggests that, under the conditions of this experiment, strain SA-1 provided the equivalent of almost 125 kg urea/ha during the growth of the rice at ambient carbon dioxide levels. The root:shoot



Figure 4. Rice shoot dry weight at final harvest as a function of CO₂ concentration and <u>Anabaena variabilis</u> cyanobacteria treatment. SA-O is the parent strain, and SA-1 is the ammonia-excreting mutant strain.





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Figure 5. Rice root dry weight at final harvest as a function of CO₂ concentration and <u>Anabaena variabilis</u> cyanobacteria treatment. SA-0 is the parent strain, and SA-1 is the ammonia-excreting mutant strain.

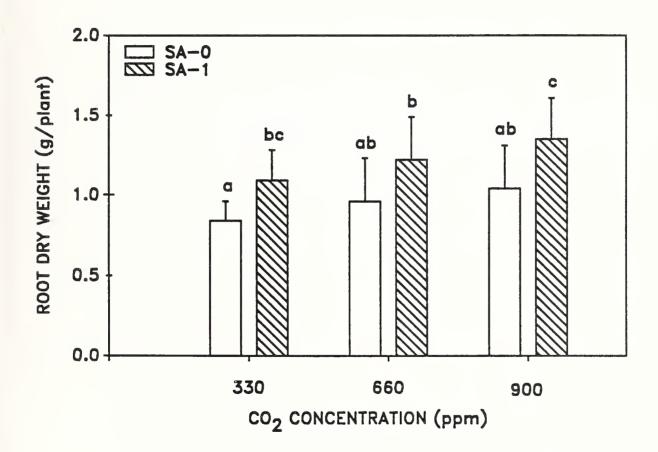


Figure 6. Rice grain dry weight at final harvest as a function of CO₂ concentration and <u>Anabaena variabilis</u> cyanobacteria treatment. SA-O is the parent strain, and SA-I is the ammonia-excreting mutant.

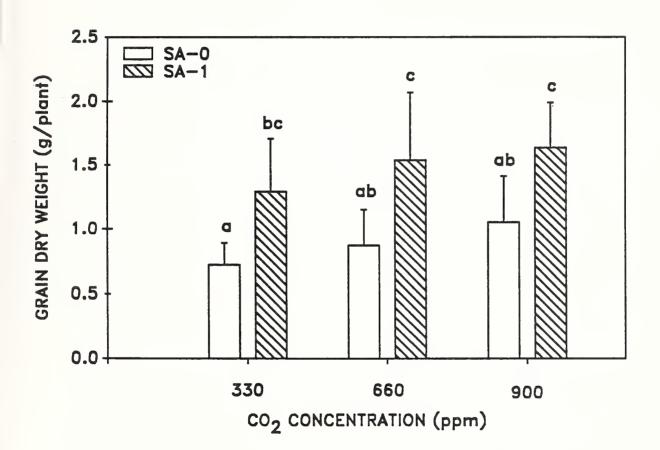
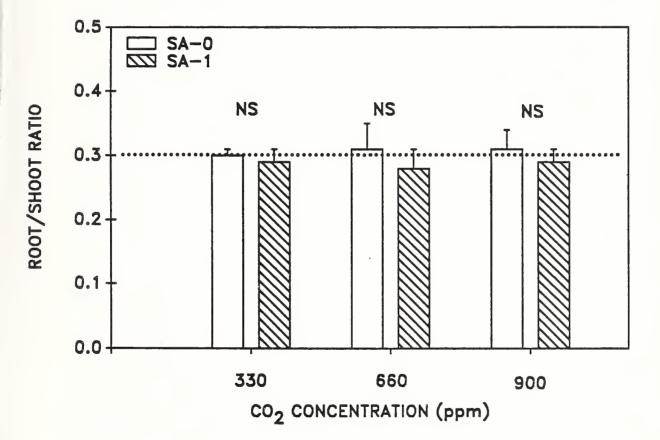


Figure 7. Rice root/shoot dry weight ratios at final harvest as a function of CO₂ concentration and Anabaena variabilis cyanobacteria treatment. SA-0 is the parent strain, and SA-1 is the ammonia-excreting mutant.



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ratios were very stable regardless of inoculation treatment or carbon dioxide concentration (Figure 7), and indicates that the biomass increases were the result increased available nitrogen in the rooting environment not the involvement of plant-like phytohormones produced by the cyanobacteria. The percent nitrogen in the plant tissue, averaged over CO, and inoculation treatment, was 0.73 \pm 0.07, 1.45 \pm 0.11 and 0.76 ± 0.07 (mean ± SD) in the shoot, grain and root material, respectively. Although the percent nitrogen in the tissue appeared greater from plants inoculated with the ammoniaexcreting strain, variability in the data statistical separation of the means. The total nitrogen content per plant showed slight, but non-significant, increases with increasing carbon dioxide enrichment. However, the total nitrogen was significantly greater (P = 0.05) in rice plants that had been inoculated with strain SA-1 when compared to tissue from plants inoculated with strain SA-0. These findings reflect the significant differences found in plant dry weight between the two inoculation strategies. These findings are consistant with previous results (1, 18, 27) that indicate strain SA-1 has the potential to stimulate the growth of rice, and possibly other crops, and that the mechanism of this stimulation is the loss of ammonia, produced by nitrogenase under photoautotrophic conditions, providing a source of nitrogen to support plant growth.

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